PLANT PHYSIOLOGY

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ERRATA

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Page 3, fifth line above footnote, for "glass" read gases.
Page 54, figure 1, for "M.P.R." (occurring twice) read M.P.H.
Page 58, line eight from bottom, for "SCHNADER" read SCHANDER.
Page 65, line 15 from bottom, for "(19)" read (20).
Page 66, line one, for "with" read which.
Page 74, line 9, for "HALL" read HALE.
Page 77, citation 17, for "51" read 15.
citation 20, for "Protoplasm" read Protoplasma.
citation 26, for "395" read 305.
citation 27, for "TUTTLE, G. U." read TUTTLE, G. M.
Page 80 dele line 2, and insert instead: "of microchemical color tests have been proposed. HANSEN (23) describes"
Page 138, line 2, for "0.50–ml." read 0.05–ml.
Page 205, citation 7, for "the Delicious apple" read apple trees.
Page 412, line ten, dele reference (OKUNUKI, 61).
Page 418, fig. 1, switch at far left in S should be double-pole, double throw, not a reversing switch.
Page 500, line six, paragraph on experimentation, insert "no" after word caused.
Page 503, line two from bottom, for "and that" read *i.e.*
Page 777, line twenty-one, dele hyphen.
Page 793, line twenty-six, period at end of line.
line twenty-seven, for "although" read Although, and insert comma at end of line.
line twenty-eight, for "This" read this.
last line, dele "ing" at beginning of line.

THIS NUMBER OF PLANT PHYSIOLOGY

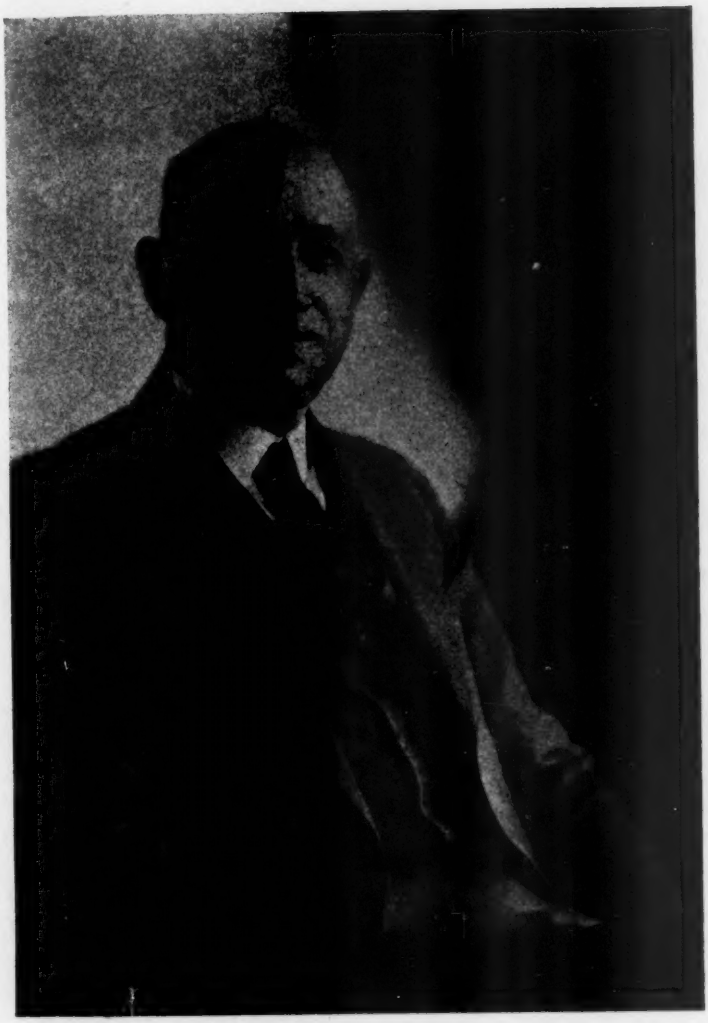
IS DEDICATED TO

RODNEY HOWARD TRUE

IN CELEBRATION OF

THE SEVENTIETH ANNIVERSARY OF HIS BIRTH

OCTOBER 14, 1866



RODNEY HOWARD TRUE
OCTOBER 14, 1866

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PLANT PHYSIOLOGY

JANUARY, 1937

DISTRIBUTION OF THE VELOCITIES OF ABSORPTION OF WATER IN THE ONION ROOT

HILDA F. ROSENE

(WITH FIVE FIGURES)

Although many investigations have been made there is, up to the present, no available study which furnishes reliable quantitative data on the distribution of the rates of water absorption in any one root. Only recently (**1, 8**) has there been any attempt to make the investigations strictly quantitative in character but all studies are open to adverse criticism since none were carried out under adequately controlled conditions. It appears that the only method at present available which provides for complete control of conditions using the *intact, uninjured,* and *unstimulated* root is that used in the present study.

Since reviews have been presented by other investigators (**1, 4, 8, 9**) no comprehensive survey of the literature will be attempted. It is important, however, to call attention to certain inadequacies of the methods employed by the more recent investigators. POPESCO (**4**) made determinations of the rates of water absorption on single roots of a number of different plants, but his results were only relatively quantitative. He employed both direct and indirect methods which involved: (1) the isolation of given regions of a root by covering these with cocoa-butter and then placing the root in a U-tube, one arm of which, drawn out to a capillary, was graduated in arbitrary units; (2) the use of dyes and microchemical reagents; and (3) plasmolysis. HÖHN (**1**) made unsuccessful attempts to use a modified form of POPESCO'S "cocoa-butter" procedure. He was unable to obtain a perfect seal with rings of cocoa-butter and he points out the impossibility of employing inelastic material to cover the region of elongation. To cover various regions of the root HÖHN substituted oil for cocoa-butter. His experiments were carefully carried out under known conditions of temperature and humidity. The chief criticism of his work is given by SIERP and

BREWIG (**8**, p. 115) who show that HÖHN's conclusions regarding the distribution of the rates of water absorption in a single root are not valid because they are based on comparative measurements of (1) different roots, and (2), on the same root, but at different time periods. Furthermore, as will be shown later, HÖHN's tabulated results do not warrant the conclusions that he gives.

URSPRUNG and BLUM (**9**), HÖHN (**1**), and SIERP and BREWIG (**8**) call attention to the limitations of all methods involving dyes and microchemical reagents, pointing out that it is impossible to determine the rates of water absorption when quantitative relations between the spread of the dyes and the movement of the water are not known. On this basis the results of POPESCO (**4**) and KELLER (**2**) employing dyes may be questioned. URSPRUNG and BLUM (**9**) based their conclusions on suction-pressure measurements. As pointed out by SIERP and BREWIG (**8**, p. 115) their method is limited because such measurements could be evaluated quantitatively only if the suction values remained constant when the root absorbed water and it has not been established that such a relation exists.

SIERP and BREWIG (**8**) used a number of micropotometers making simultaneous measurements on the same root. They state that so far as they know this was the first time such a procedure had been followed. However, the method employed in the present investigation, which also involved simultaneous measurements by several micropotometers on the same root, was first reported by ROSENE and LUND in 1934 (**6**). The technique followed by SIERP and BREWIG has serious limitations. They made measurements on the roots of plants which were in an inverted position with respect to gravity, thus producing conditions of stimulation. In order to insure water-tight connections they wound thread impregnated with wool fat around the root. They maintain that after the experiment no injury to the root was apparent but they do not give the criteria or tests upon which their conclusion was based. Wrapping the roots with thread in this manner would produce mechanical stimulation and would interfere with respiratory activities. The quantitative determinations made by HÖHN, and by SIERP and BREWIG, were expressed in milligrams per linear length, assuming that the diameter of the root was uniform throughout its length. It is obvious that such a technique furnishes data which is only relatively quantitative. Of far greater value are reliable data on the rate of absorption per unit of surface area.

Determination of the distribution of the velocities of absorption of water in roots should therefore be made on intact, uninjured, unstimulated roots, and under conditions which permit careful control of such conditions as temperature, light, oxygen, and humidity. It should be possible to make simultaneous measurements of water absorption by different regions, and

to determine the rates of absorption per unit of surface area. It is believed that the present technique fulfills these requirements.

Apparatus and method

The experiments were conducted at room temperature (25° C.) in a basement room, the temperature of which did not vary more than 2° during the longest experiments, which were of several days' duration. The observations were made on the roots of *Allium cepa,* which for the most part were grown either in tap water, or in a saturated atmosphere in vessels through which air was continuously passed.[1] In certain experiments, observations were made on roots which had developed when a given onion was growing in the experimental apparatus. Several experimental chambers were constructed, making it possible to run controls while varying the conditions in one or more chambers.

The details of one experimental chamber are shown in figure 1. The removable glass cover (A, fig. 1) rests in a groove (F) in the transite base (B) which is attached to a rack and pinion stand providing vertical adjustment for the base and cover. The base has four perforations lined with bakelite tubing through which connections pass from the interior to the exterior of the chamber. The onion bulb rests upon a cork ring which is placed in a hole in a swivel attachment (C). The swivel attachment is supported by the glass tube (G), which is cemented to a brass rod (D). The brass rod passes through a perforation and is supported on the outside by a three-way mechanical micromanipulator. With this arrangement the onion bulb may be moved to any desired position by manipulation from the outside. Each micropotometer (H) consists of a calibrated glass tube made from carefully selected millimeter pyrex tubing of uniform bore. One end of each tube (a, b, c, d, fig. 1) was ground down on two opposite sides of the bore and a hole was bored through to permit passage of the root. One or more micropotometers were cemented with DeKhotinsky to a glass rod (E) which was supported by a three-way mechanical manipulator which provided delicate adjustments and accurate placing of the micropotometers around the root. The potometers varied in length from 25 to 40 millimeters but were the same for any one experiment. Glass tubes (I and O), which pass through rubber stoppers fitted into perforations of the base, provide for inlet and outlet of glass. Moisture and airtight seals at the perforations through which the rods (D and E) passed were made with finger stalls designated as M in figure 1. These were filled with water. By packing plasticene around the base of the chamber where it fits into the groove, the interior could be completely sealed off from the exterior.

[1] The experiments reported in this paper were carried out in the fall of 1934 on onion sets grown the preceding spring. Observations on onion sets procured this spring (1935) show slightly higher rates of water absorption.

Throughout all of the experiments reported in this paper, air saturated with moisture was continuously passed through the chamber. Both inlet and outlet tubes (I and O) were connected to wash bottles. The latter connection served as a test to determine any leakage of air through the apparatus and as a means of gauging the relative rate of gas flow by counting the bubbles per unit time.

Most of the experiments were made on a single intact root after the sister roots had been cut off. Removing the onion from the vessel in which it grew, cutting the sister roots, and placing it in the experimental chamber, required such a short period of exposure that the root had no time to dry out in any region. This was determined by microscopic examination in a

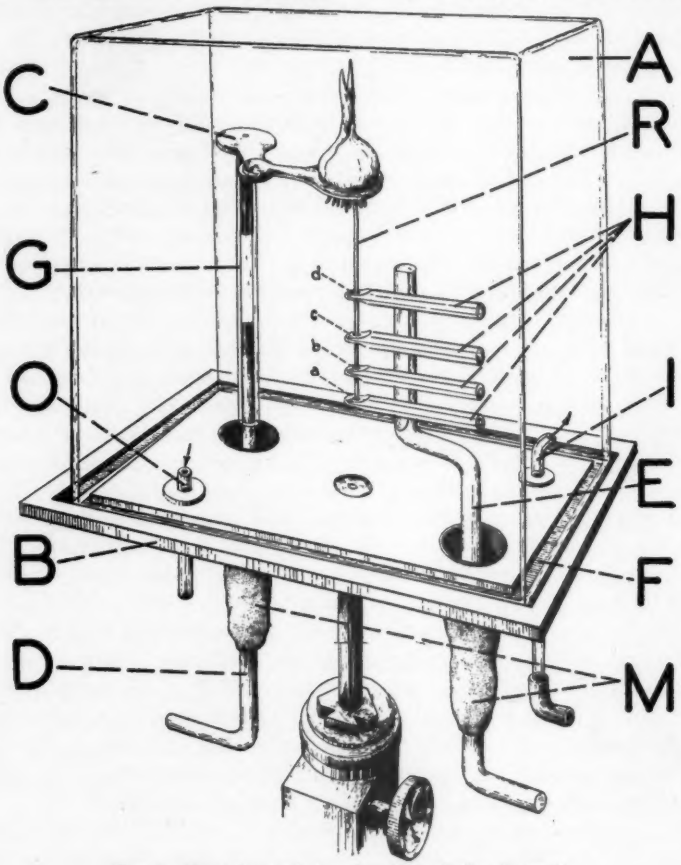


FIG. 1. Experimental chamber (description in text).

series of experiments to determine whether or not drying appeared. The root (R, fig. 1), was threaded through the openings (a, b, c, d) of the micropotometers by adjustments made with the micromanipulators on the outside, lowering the brass rod (D) and passing the root down through the openings; or by raising the glass rod (E) and passing the micropotometer tubes up over the root to the desired positions. All adjustments of the mechanical micromanipulators and all measurements were made with the aid of a horizontal microscope which was fitted with a micrometer ocular. By this means very delicate adjustments of the positions of the micropotometers could be made without mechanical injury. By raising the bulb support (D), or lowering the micropotometer support (E), at frequent intervals throughout an experiment it was possible to maintain the positions of the micropotometers at a given distance from the root tip as elongation took place. The quantity of water absorbed was determined by making consecutive minute measurements of the movement of the terminal meniscus in each tube. The height of the water column at each region was determined by measuring the distance between the upper and lower menisci around the root where it passed through the micropotometer. There was no flow of water down the root and after a period of an hour or more, depending upon the height of the water column at the beginning, the distance between the lower and upper menisci at each micropotometer contact reached a steady value which was maintained relatively constant. At the apex, where increase in diameter took place, there was a slight shift in the height of the water column. Determinations of the average diameter and height of the water column at each contact were made at frequent intervals, depending upon the nature of the experiment. No measurements were made during the first 5 to 10 hours after the onion had been placed in the chamber, in order to permit it to come into flux equilibrium with the conditions of the environment. Favorable conditions for growth were present in the experimental chamber, for the roots and leaves of the onions grew in a normal manner. New roots appeared and developed and these were sometimes used for observation. No root hairs were observed on any of the roots, whether grown in moist air, or in tap water.

Measurements were made in millimeters to the second decimal place. Although great precautions were taken when readings were made, and although the menisci at each contact appeared clear-cut and remained fairly steady after flux equilibrium conditions were reached, it might be that the absolute height of the water column was not observed. Variations of the diameters at the upper and lower menisci at a single contact did not exceed 0.02 millimeter. In all of the experiments during which the micropotometers were kept at relatively constant positions with respect to the root tip by lowering them at frequent intervals, the absolute distance from the apex

varied from one reading to the next. When adjustments were made at intervals of fifteen minutes the variation did not exceed 0.11 mm., except in a very rapidly growing root, where it reached 0.2 mm. When the interval of adjustment was longer, the variation was, of course, greater.

Investigation

A. Absorption of water by apical regions

1. Rates of absorption in relatively young roots 25 to 35 mm. in length

The quantity of tap water absorbed from 6:30 A.M. one day to 2:30 A.M. the day following, by four apical regions of the root exhibiting considerable diversity of morphological character, is shown in figure 2. The bulb with

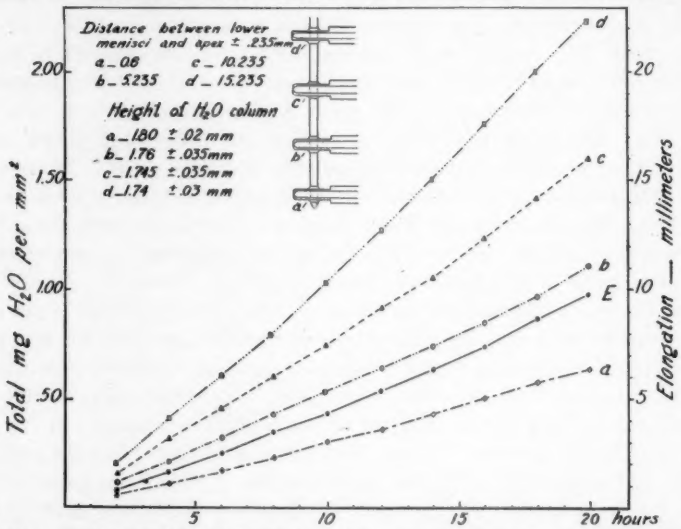


Fig. 2. Water absorbed by 4 apical regions of a single root with initial length of 25 mm. Curves *a*, *b*, *c*, and *d* show mg. of water absorbed per mm.² of absorbing surface at the micropotometer contacts *a'*, *b'*, *c'*, *d'* shown in the root diagram. Initial diameters at *a'*, *b'*, *c'* and *d'* are 0.710, 0.735, 0.740, and 0.752 mm. respectively. Curve *E* shows elongation in mm.

three leaves 4 to 6 cm. in length and a single remaining root had been placed in the chamber with the micropotometers in position the night before the determinations were made. At 6:30 A.M., by means of the mechanical micromanipulators on the outside, the micropotometers were lowered until the position of each was respectively 0.4, 5.0, 10.0, and 15.0 mm. from the apical point of the root. Every half hour or less, the micropotometers were lowered with respect to the growing root in order to maintain them in the

same relative positions. Measurements of the root diameters at each meniscus of the water columns, the height of the water column at each region, the movement of the terminal meniscus in the micropotometer, and elongation were recorded every four hours. The height of each water column with the maximum variations between readings, the distance between the lower meniscus at each micropotometer and the apex, and the average diameter of each contact are given in figure 2. Elongation is shown by curve *E*. The rate of elongation is fairly uniform, tending to increase during the night, especially after midnight. Curves *a*, *b*, *c*, and *d* (fig. 2), show the milligrams of water absorbed, calculated in terms of unit surface exposed to the water of each micropotometer.

Curve *a* represents the quantity of water absorbed by the surface in a region where active cell division is taking place, and tissue differentiation is at a minimum; curve *b* shows the milligrams of water absorbed by the surface in the fifth and sixth millimeters which is the zone just proximal to the region of elongation, and curves *c* and *d* give the quantity of water absorbed by regions relatively older where tissue differentiation has taken place and vacuolization tends to reach a maximum.

The curves clearly show that water was absorbed by the surface in all four of the regions mentioned above, and that the rates of absorption in the regions were very different. Greater quantities of water were absorbed by the relatively more basal regions. A comparison of the slopes of all of the curves shows that there was an increase in the rate of absorption in each region with time, the most rapid rates appearing toward the end of the experiment. In this experiment, which is typical, the greatest increase in the velocity of absorption of any one zone occurred in the region which is in the 15th and 16th millimeter from the apical point of the root.

When all of the roots are left intact, the quantity of water absorbed by any region of one of the roots is less than if all of the roots but one are cut off. This fact is shown by the curves in figure 3, which give typical results obtained when measurements were made on a given root with (1) all of the sister roots intact and (2) all of the sister roots removed. In this case, the observations were made on a bulb which had three leaves 3 to 5 cm. in length, and fourteen roots 10 to 30 mm. long. Three micropotometers were placed on a root which was 27.3 mm. in length in the positions designated as x', y', z' in diagram N (fig. 3). These positions corresponded to the regions (1) where active cell division was taking place; (2) where elongation was at a maximum; and (3) the zone proximal to the region of elongation. No observations were made until 8 hours after the bulb had been placed in the chamber. From then on, measurements were made every four hours, and adjustment for elongation was made at half-hour intervals except during the night. The two morning readings, therefore, showed

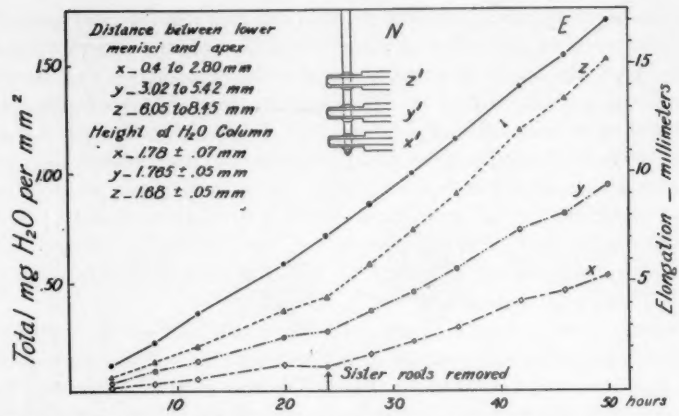


FIG. 3. Absorption of water by 3 apical regions of a single root when (a) all the roots were intact and (b) the sister roots were removed. Curves x, y and z show the mg. of water absorbed at the contacts of the micropotometers x', y' and z' illustrated in diagram N. Diameters at contacts of the micropotometers x', y', z' were 0.77, 0.787 and 0.80 mm. respectively. Curve *E* shows elongation. The arrow on the base line indicates time at which the sister roots were removed.

higher values since these represented the absorption of water during an interval when the positions of the micropotometers relative to the base varied from 2 to 2.5 mm. After 24 hours, as designated by the arrow on the base line in figure 3, all of the roots but the one which was in contact with the water in the micropotometers, were quickly cut off with sharp scissors without changing the positions or the terminal menisci of the micropotometers in any way. The heights of the water columns are tabulated in figure 3. Maximum variation during any one interval was 0.07 mm. which occurred at the apical micropotometer the second night. The limits of variation in the positions of the micropotometers relative to the apex of the root, and the average diameter at each contact, are also tabulated in figure 3. As shown by curves *x*, *y*, and *z* (fig. 3) tap water was absorbed by the surfaces in all three of the regions mentioned above. Pronounced change in the slopes of the latter half of each curve demonstrates that after the sister roots had been removed, greater quantities of water were absorbed by all of the surfaces exposed to tap water in the micropotometers. Ten other experiments similar to the above, with the micropotometers in various positions, showed essentially the same results. In each case, the rate of absorption in the single intact root was greater after removal of the sister roots, and the greatest quantity of water was absorbed by the region nearest the base. In all of the experiments in which measurements of the absorption of water were made on a single intact root with the sister roots removed,

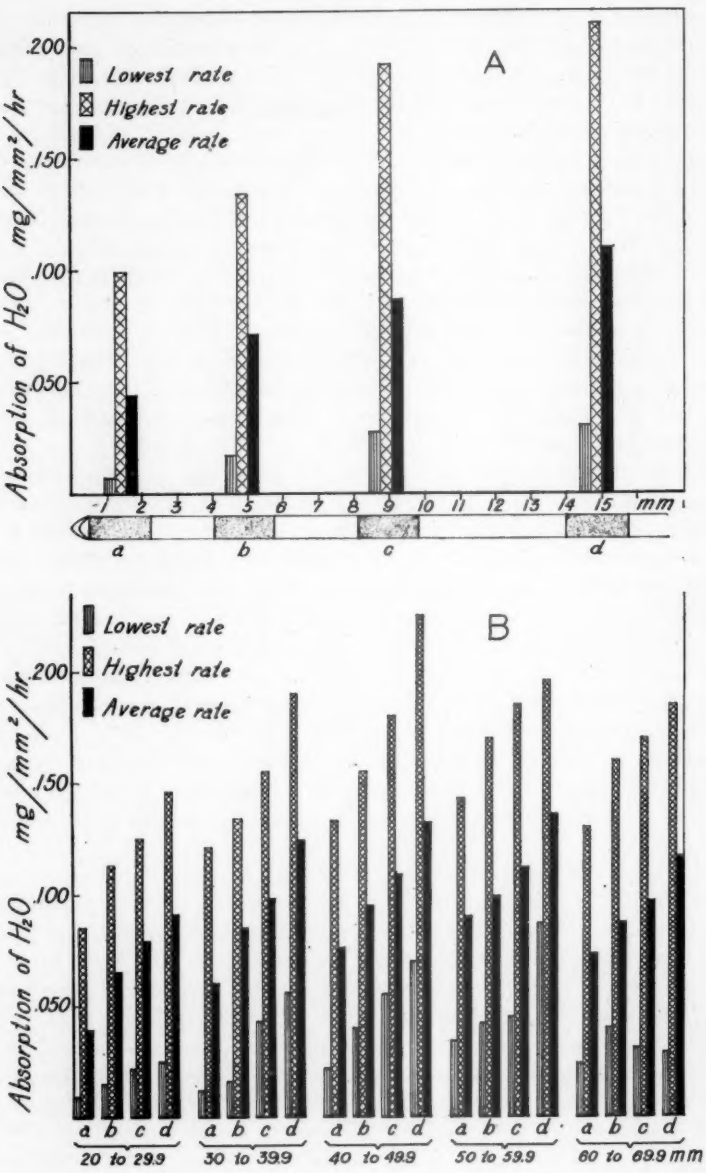
the observed rates of absorption were relatively higher than when the sister roots were present, but the distribution of the velocities of absorption remained the same. Changes in the distribution of the rates of absorption occurred but these were related to other phenomena, not to the presence or absence of sister roots.

By using a greater number of micropotometers, by placing them close together, and by changing the positions in different experiments, it was found that all regions of the root between the root cap and 16 mm. from the apex absorbed water. The highest rates observed were exhibited by the region 14 to 16 mm. from the apex. All of the roots exhibited a distinct unidirectional gradient of increase in the rates of water absorption by unit surface areas from the apex towards the base. However, individual roots show considerable diversity in water-absorbing power. The results of measurements made on 10 roots with initial lengths of 25 to 32 millimeters, continuing throughout a fourteen-hour observation period, and using four micropotometers are given in figure 4, A.

Frequent adjustments (15 to 30 minute intervals) of the micropotometers were made in order to maintain them in the positions designated by the stippled areas (*a, b, c, d*) in the diagram of the root illustrated in figure 4. The greatest variation in any one experiment from the regions designated was 0.5 mm. in a rapidly growing root. Measurements were made every two hours, and calculations were made of the average rate of absorption in milligrams of water per hour per square millimeter of surface exposed to the water in the micropotometers. The highest and lowest rates given in graphic form in figure 4 A, are the rates of absorption in four regions of two different roots and are the result of averaging seven determinations throughout the fourteen-hour period. On the other hand, the graphic representation of the average rates in figure 4 A, are the average rates of all measurements made on the designated regions in 10 different roots.

Several interesting facts are apparent when a comparison is made of the heights of the bars in figure 4 A. In the first place, the comparison shows that there is a wide variation between the highest and lowest rates observed in any one region; in the second place, it shows that in spite of the large difference between these two roots which exhibit the highest and lowest rates, each individual root manifests a distinct functional polarity with respect to the distribution of the rates of absorption in these regions; and in the third place, the polar distribution of the rates of water absorption is also shown by comparing the average rates of all of the regions in all of the roots.

A comparison of the percentage differences in the average rates of absorption of the four regions in all of the roots shows that the region between 14 and 16 millimeters from the apex absorbed water at a rate which


A
Lowest rate
Highest rate
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.150
.100
.050
a b c d
20 to 29.9
30 to 39.9
40 to 49.9
50 to 59.9
60 to 69.9 mm

was 26.4 per cent. greater than the region between 8 and 10 millimeters, 54.9 per cent. greater than the region between 4 and 6 millimeters, and 150 per cent. greater than the region 0.5 to 2.5 millimeters from the apex. The region between 8 and 10 millimeters absorbed water at a rate which was 22.5 per cent. greater than the rate in the region between 4 and 6 millimeters, and 17.7 per cent. greater than the region at the extreme apex; and the zone between 4 and 5 millimeters absorbed water at a rate 61.3 per cent. greater than the most apical region designated as *a* in the diagram of the root in figure 4 A. These four regions were selected for special study because they are the regions which determine the characteristic distribution of bioelectric potential in the onion root.

2. Effect of age on the rates of absorption in apical regions

In order to determine the effect of age, measurements of the rate of absorption were made throughout a growth period when the roots elongated from an initial length of 20 mm. to 90 mm. or more. The micropotometers were maintained in the relative positions designated as *a*, *b*, *c*, and *d* in the diagram of the root (fig. 4 A) by moving them at intervals of an hour during the day and 6 hours at night. The values obtained at the first morning determinations were therefore a little high. The greatest shift in the position of the micropotometers in a single root was 4 millimeters, the average for all roots, 2.4 millimeters. The micropotometers had to be refilled during the course of the experiments but this was done very quickly with a pipette and no apparent drying of the root occurred.[2] Measurements were made every 4 to 6 hours. In order to compare the change in the rate of absorption of each region with age, the average rates of each region in each root during a growth interval in which the root increased in length from 9 to 9.9 mm. (to be designated as a 10-mm. growth period) were determined. Measure-

[2] Recent modifications of the apparatus permit refilling of the micropotometers, and removing any collected fog on the glass wall facing the microscope, without changing the humidity of the interior of the chamber.

Fig. 4. Comparative rates of absorption in different roots. A. Rates of water absorbed by 4 apical regions of 10 different roots during a 14-hour observation period. Initial lengths of roots were 25 to 32 mm. The positions of the micropotometers are indicated by the stippled areas *a*, *b*, *c*, and *d*, in the diagram of the root while the vertical bars above represent rates of absorption in the corresponding region. Bars with vertical lines give rates of absorption in the root which manifested the lowest rate, bars with crossed lines give rates of absorption in the root which manifested the highest rate (each bar is average of 7 observations). Solid black bars give average rates in the designated regions during the 14-hour period for all roots. B. Change in rate of water absorption with age. Vertical bars represent the rates of absorption during 5 growth intervals by each region corresponding to *a*, *b*, *c*, and *d* in the diagram of the root. Bars with vertical lines and crossed lines give lowest and highest rates respectively, solid black bars give average rates of 11 different roots. Growth intervals are designated in mm. below the vertical bars.

ments were made on many roots. The exact time at which the 9 to 9.9 mm. increase in length was reached was not observed in all cases, since on certain nights the time interval from one reading to the next was 6 hours. Only the results obtained from eleven roots, in which the transitional changes from one growth interval to the next were observed, are included in the graphic representation in figure 4 B. Tables of detailed measurements are omitted in order to save space.

The highest and lowest average rates observed in individual roots during the 10-millimeter growth period are represented graphically in figure 4 B by the vertical bars with the crossed lines, and with the vertical lines, respectively. A comparison of the heights of the bars with vertical lines shows that in the corresponding root the increase in rate of absorption of each region for each 10-mm. growth period was not uniform. Regions *a* and *b* in this instance reached a maximum rate during the 50 to 59.9-mm. growth period, region *c* in the 40 to 49.9-mm. growth interval, and region *d* in the 50 to 59.9-mm. interval. A comparison of the heights of the crossed bars show that in another individual root region *d*, which was 14 to 16 mm. from the apex, reached a maximum earliest (40 to 49.9-mm. interval), and regions *c*, *b*, and *a* followed in the next interval (50 to 59.9-mm.). The results obtained from measurements on these two roots demonstrate that individual variations exist and that a given region does not necessarily exhibit a maximum rate of absorption when another region (or regions) of the same root reaches a maximum rate. However, characteristic changes are the rule. The majority of roots tend to manifest maximum absorption rates when they reach lengths between 40 and 60 mm. as shown by a comparison of the heights of the solid black bars in figure 3 B. These represent the average rates of absorption in the designated regions for all of the roots during five growth intervals. Since during the night there was a relatively greater increase in the distance of each water column from the apex, this would tend to make the average rates of absorption somewhat higher at each region than if the positions of the micropotometers relative to the apical point did not change. Exceptions were found, especially in relatively short roots (less than 40 mm.) which developed late and grew slowly, and in relatively long roots (over 60 mm.) which developed early and grew rapidly. Calculations of the percentage difference in the average rates of absorption in each region for all the growth periods are tabulated below.

1. Region *d* which was 14 to 16 millimeters from the apex.

Growth intervals in mm.	Percentage differences
20 to 29.9 and 30 to 39.9	36.0 per cent. increase
30 to 39.9 and 40 to 49.9	6.4 per cent. increase
40 to 49.9 and 50 to 59.9	3.0 per cent. increase
50 to 59.9 and 60 to 69.9	16.2 per cent. decrease

2. Region *c* which was 8 to 10 millimeters from the apex.

Growth intervals in mm.	Percentage differences
20 to 29.9 and 30 to 39.9	24.1 per cent. increase
30 to 39.9 and 40 to 49.9	11.2 per cent. increase
40 to 49.9 and 50 to 59.9	2.75 per cent. increase
50 to 59.9 and 60 to 69.9	15.4 per cent. decrease

3. Region *b* which was 4 to 6 millimeters from the apex.

Growth intervals in mm.	Percentage differences
20 to 29.9 and 30 to 39.9	30.7 per cent. increase
30 to 39.9 and 40 to 49.9	11.7 per cent. increase
40 to 49.9 and 50 to 59.9	4.2 per cent. increase
50 to 59.9 and 60 to 69.9	13.7 per cent. decrease

4. Region *a* which was 0.5 to 2.5 millimeters from the apex.

Growth intervals in mm.	Percentage differences
20 to 29.9 and 30 to 39.9	53.8 per cent. increase
30 to 39.9 and 40 to 49.9	25.0 per cent. increase
40 to 49.9 and 50 to 59.9	18.4 per cent. increase
50 to 59.9 and 60 to 69.9	23.2 per cent decrease

B. Absorption of water by relatively more basal regions

By placing a large number of micropotometers on a given root, and by shifting the positions of the micropotometers when desired, determinations of the rates of absorption in all the root regions between the root cap and the bulb were made. It was found that all the root regions from the root cap to the base absorbed water, but at different rates, and that the distribution of the rates of absorption was not the same for all roots. The determinations demonstrated that in roots less than 50 mm. in length, there was in general an increase in the rate of absorption per unit surface from apex to base, and the highest rates occurred in the regions nearest the bulb; whereas in roots over 70 mm. in length, the regions of maximum absorption were relatively more apical. In the majority of roots over 80 mm. in length, maximum rates were observed in regions within the first 40 mm. from the apex. Although experiments have been made on many roots of different lengths and age, it was found that all of the root regions between the root cap and the base absorbed water. The longest root studied was 220 mm. in length. Most roots tend to develop lateral roots at the age of 2 to 3 weeks. It was observed that there was a definite shift of the region of maximum absorption rate towards the apex, with the development of lateral roots. It is entirely possible and highly probable that the regions of the

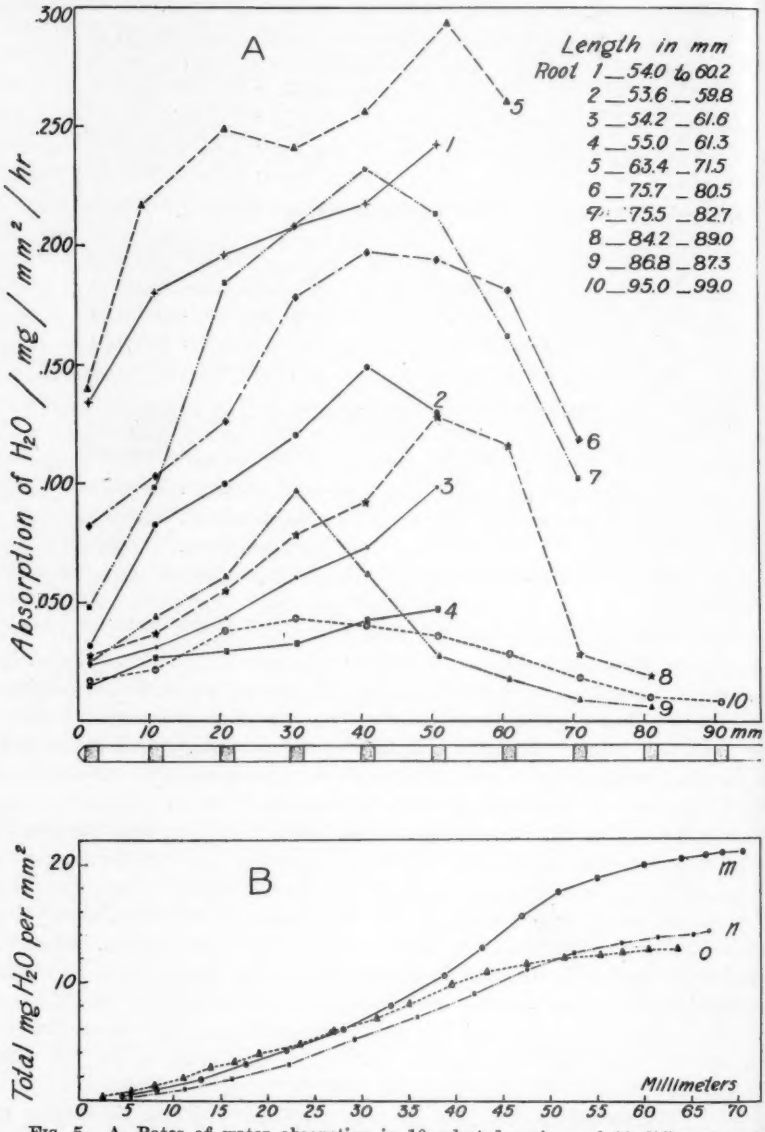

FIG. 5. A. Rates of water absorption in 10 selected regions of 10 different roots during a 12-hour observation period. Curves 1 to 10 represent the average rates of water absorption by 10 different regions in ten different roots corresponding to the numbers on

root not exposed to the water of the micropotometers also absorbed water since they were in a saturated atmosphere.

Figure 5 A shows the rates of water absorption in selected regions of ten roots, the initial lengths of which varied from 54 to 95 mm. The positions of the micropotometers were maintained relatively constant with respect to the apex of each root by moving them at intervals of one-half hour or less. Measurements were made every two hours during a 12-hour period, and the average rate of absorption for each 12-hour period was calculated and plotted on a graph as shown in figure 5 A. The initial and final lengths of each root are given in figure 5 A, after the first column of numbers, which correspond to those of the curves of distribution. The stippled areas in the diagram of the root below the curves represent the positions of the micropotometers which were placed 10 mm. apart. A comparison of all of the curves shows that the highest rates of absorption are exhibited by relatively more basal regions, and that the region where maximum rates occur varies in individual roots, but is closely linked with the lengths of the root. The initial lengths of the 4 roots which exhibited the rates given by curves 1, 2, 3, and 4 (fig. 5), were less than 60 millimeters. A comparison of the slopes of these curves demonstrates considerable individual differences in the rates of absorption of different roots, but all manifest an increase in the rate per unit surface from apex to base. Note that in curve 5 (fig. 4 A) there are variations in the distribution of the rates of water absorption, although the highest rates relative to the apex are observed in the basal regions. As shown by curves 1, 2, 3 and 4, maximum rates of absorption are found at or very near the base in roots of this length. These roots were 10 to 15 days old. Curves 5, 6, 7 (fig. 5 A), which represent measurements on roots 63 to 75 mm. in length, show that comparatively rapid rates of absorption are exhibited in all regions and that the maximum rate appears in regions nearer the apex. The experiments show that, with increasing age, the regions of maximum absorption shift toward the apex. This fact is further emphasized by comparing curves 1 to 8 with curves 8, 9, and 10, obtained from experiments on roots over 80 mm. long.

The above results and those depicted in figure 4 B indicate that each root region exhibits an increase in the rate of absorption, which goes through a maximum with age. Experiments to prove this were made, and the results are given by curves *m*, *n*, and *o* (fig. 5 B). Each curve represents the

the right of the figure. The corresponding initial (first column) and final lengths (second column) of each root are given after the root numbers. Stippled areas of the root in the diagram below the curves show the positions of the micropotometers. B. Change in quantity of H_2O absorbed by a given region with age. Curves m, n, and o obtained from 3 different roots show the mg. of water absorbed by a single region during an observation period when the distance between the fixed micropotometer and the apex increased with growth.

rates of absorption in one region of a single root. The initial lengths of the roots which correspond to curves *m*, *n*, and *o*, were respectively 20.3, 27.0, and 32.6 millimeters. In each experiment, after the onion bulb had been in the chamber 8 hours, the position of the single micropotometer used was adjusted so that the lower meniscus was within 0.5 mm. of the root tip. Measurements were made at 10-hour intervals. Since the position of the micropotometer on each root was not changed, the distance between it and the growing root tip increased with increase in root length as the root grew, but remained the same distance relative to the base of the root, after fifteen hours or less, depending upon the rate of elongation. The slopes of the curves *m*, *n*, and *o*, all show a typical sigmoid curve. It is obvious that at each region there was an increase in the rate of absorption with age, and that each region passed through a maximum when it was from 40 to 60 mm. from the apex. These experiments corroborate the conclusions given above that the distribution of the velocities of absorption depends upon the age of the root.

Discussion

The experimental evidence which has been presented heretofore regarding the absorption of water by the apical meristem is not in agreement. PRIESTLEY and TUPPER-CAREY (5) showed that the apex was relatively impermeable, HÖHN (1) that comparatively little absorption occurred, while SIERP and BREWIG (8) maintained that the first 5 mm. of the apex not only showed an absence of absorption, but that it actually gave off water. A large number of investigators, including POPESCO (4), URSPRUNG and BLUM (9), KELLER (2), and HÖHN (1), claim that the chief absorbing zone is the region just proximal to the zone of elongation. KELLER (2) maintains that the distribution of the rates of water absorption in the root corresponds to the distribution of bioelectric potentials. Most of the work on absorption rates has been carried out on roots other than those of the onion. Just as individual roots exhibit variations in the velocities of absorption and their distribution, it is to be expected that differences will be found in the roots of different plant species. However, such differences would not account for the pronounced lack of agreement found among the investigators mentioned above.

It is doubtful if much value can be placed on the recent experiments carried out by SIERP and BREWIG (8) since the roots were stimulated by inverting the normal orientation with respect to gravity and by wrapping the roots at intervals with cotton thread. It does not seem likely that the apical meristem gave off (excreted?) water unless disintegration of the tip occurred. The authors state (8, p. 107) that the increase in the micropotometer volume was at least 149 $mm.^3$ whereas the increase in the volume of the root apex resulted in a maximum of 3 $mm.^3$ but they do not state how

they determined the increase in root volume. Measurements have been made by the author on the roots of onions maintained in the inverted position but no such unique phenomenon has been observed. On the contrary, the regions between the root cap and base all absorbed water and the polar distribution of the rates of absorption was maintained. However, in these experiments, transpiration was at a minimum. SIERP and BREWIG on the other hand, enclosed the inverted stems of their plants in a chamber and varied the humidity.

SIERP and BREWIG also state that, with increase in transpiration rates, there is a migration of the region of maximum absorption toward the apex. They do not, however, show whether this shift is dependent on age, as it is in the onion root. Owing to the fact that the onion bulb provides a reservoir of water, the onion does not furnish good material to determine the relations between water absorption and transpiration. Nevertheless the writer found that in the onion root there was an increase in absorption at all of the root regions under certain conditions when transpiration was increased; but the polar distribution of the rates of water absorption was always maintained.

HÖHN (**1**) claims that the entire root surface of the roots he studied (*Zea mays, Triticum vulgare, Tradescantia fluminensis*) absorbed water. As mentioned above, he failed to make simultaneous measurements of water absorption in a single root, and his tabulated data do not support the conclusions he has made. HÖHN maintains that the hourly water absorption of an old root, calculated on the basis of unit length, is greater than that of a younger root in most of his experiments; but his tables do not warrant such an interpretation. Table 8 (**1**, p. 550) shows, for example, that a root 65 mm. in length absorbed 0.43 mg. of water per hour per mm. length of the root, whereas a root less than half this length (26 mm.) absorbed 0.89 mg. of water per hour per unit root length. It may be that the shorter root had grown slowly and was actually the older root, but HÖHN presents no evidence in favor of this suggestion. Table 9 (**1**, p. 551) shows that a root 58 mm. in length absorbed 0.62 mg. of water per unit length in unit time, while another root 25 mm. in length absorbed 1.16 mg. of water. The longer root, which was over twice the length of the shorter, absorbed water at half the rate. HÖHN's tables merely establish the fact that individual differences exist with respect to the rates at which different roots absorb water. Although HÖHN states that he used "similar" roots, his data furnish no evidence in support of his statements, since no measurements of root diameter were made.

KELLER (**2**) used dyes to determine the rates of water absorption in the root, and reached conclusions similar to those of POPESCO (**4**). In favor of his hypothesis that electro-osmotic forces are involved in water absorption and transport, KELLER calls attention to the correspondence of their

results—which maintain that the main absorbing region of the root is the region of elongation—with those of LUND (3) on bioelectric potentials in the onion root, which show that the region of elongation is electronegative to other regions of the root.

The results presented in this paper of direct quantitative determination of the rates of water absorption in the onion root itself show that there is no correspondence between the unidirectional gradient of distribution of the velocities of water absorption in roots less than 50 millimeters in length, and the distribution of electric potentials in roots of similar length as shown by LUND and co-workers (3, 7). The region of relatively low electronegativity from 4.5 mm. to between 7.5 to 14 mm. is not the region which exhibits either a maximum or minimum rate of absorption except in very old roots (over 3 weeks) and then rarely. The fact that the gradient of the distribution of electric potentials in the onion root does not correspond to the gradient of the distribution of velocities of water absorption does not necessarily indicate that electric energy is not utilized in the processes of absorption and transport. Experiments to determine possible linkage between the production of electric energy and the absorption of water in the onion root are being carried out at the present time.

The most striking facts brought out in this paper are that in the onion root, all root regions between the root cap and the base absorb water at different rates, that the rates of absorption per unit area change with time, going through a maximum. The precise measurements show definitely that the distance from the apex to the exact region which exhibits the highest rate of absorption differs in individual roots and changes with age.

The present paper makes no attempt to consider the mechanism or mechanisms involved in the absorption of water; but it furnishes for the first time precise quantitative data concerning the distribution of the velocities of water absorption in a single intact root under carefully controlled conditions.

Summary

1. A technique is described by means of which precise quantitative data on the velocities of absorption of tap water by different root regions of the same intact root may be simultaneously determined under carefully controlled conditions.

2. All root regions of the onion root between the root cap (on which no measurements were made) and the bulb absorb tap water but at unequal rates.

3. In relatively young roots (less than 50 mm.) there is a unidirectional gradient of the distribution of velocities of water absorption, the region of maximum absorption appearing at the base.

4. In relatively older roots (more than 70 mm.) there exist two pronounced unidirectional gradients with maximum absorption velocities appearing in regions 40 to 60 mm. from the apex.

5. The velocities of absorption of water are less in all root regions between the root cap and base in any one root when all of the sister roots are present. Removal of sister roots increases the rates of absorption in all root regions of the remaining root.

6. Each root region goes through a maximum with respect to age, exhibiting highest velocity rates when the root is from 40 to 60 mm. in length.

7. With increase in age there is a shift of the region exhibiting maximum rates toward the apex.

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RELATION OF NUTRIENT SALT CONCENTRATION TO GROWTH OF THE TOMATO AND TO THE INCIDENCE OF BLOSSOM-END ROT OF THE FRUIT[1]

W. Rei Robbins

(WITH EIGHT FIGURES)

Introduction

The control of the nutrient salt concentration of the solution bathing plant roots is a factor of importance in plant growth whether in artificial culture or in soil. When essential salts are present in very low concentrations, growth may be limited by a deficiency of one or more of the elements necessary in plant metabolism. When salt concentrations are high, growth is dependent upon factors other than mere quantitative nutrient supply. One of these factors is the osmotic concentration of the solution which, in turn, affects the water relations within the plant.

In the tomato plant, a serious disturbance of the normally existing water relations has been assigned as the cause of the physiological disease of the fruit known as blossom-end rot. This disease consists of the development at the stylar end of the fruit of a spot having a slight, watery discoloration of the tissue which increases in size and gradually turns from brown to black. It is accompanied by a shrinkage of the affected part of the fruit. The symptoms of this disease are known, its occurrence has been frequently described, and the serious loss which it causes in some seasons is well recognized (**1, 3, 15, 28, 29, 35**).

Although the development of blossom-end rot is evidently concerned with the adequacy of the water supply of the affected tissues of the fruit, yet many other factors than the concentration of the nutrient solution have been indirectly associated with the incidence of this fruit disorder in the garden, field, and greenhouse. Low soil moisture has been the factor most often associated with the development of blossom-end rot of the tomato (**3, 29, 35**). Various other cultural or environmental factors have likewise been associated with the incidence of this disease as: over-watering the soil (**15**), and the attendant lack of gaseous exchange in the soil following heavy watering (**28**); the staking of tomato plants (**29, 30**); the use of unbalanced fertilizers high in potassium and ammonium salts (**1, 35**); and the transfer of tomato plants from a shaded to a non-shaded greenhouse (**3**). Vigorously vegetative plants have been found to be more generally affected with blossom-end rot than plants grown less vigorously (**3, 15**). On the other hand,

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shading the plants (**35**), and the use of nitrate rather than ammonium nitrogen salts as constituents of the fertilizer have been thought to reduce the incidence of blossom-end rot (**3, 29, 35**).

The idea has been suggested (**35**), that this malady is related to transpiration and that there seems to be competition between the fruits and the leaves for water, and that when wilting of the leaves begins, water may then be drawn from the fruits. In fact, WILSON and RUNNELS (**36, 37**) have shown that the well known increase in the rate of transpiration accompanying the application of a Bordeaux mixture spray was associated with an increased percentage of fruits showing blossom-end rot compared with that of unsprayed plants. On the other hand, they found that a heavy oil spray reduced both the transpiration and the percentage of fruits showing blossom-end rot as compared with that on unsprayed plants. However, there seems to be no general appreciation of the relation between the concentration of the solution surrounding the roots and the incidence of blossom-end rot of the tomato fruits.

The object of this paper, therefore, is to study the relation of the salt concentration of the nutrient solution to the growth of the tomato plant and to the incidence of blossom-end rot of the fruit, and to show how various other factors of nutrition and environment may be important in modifying these relations.

Materials and methods

The general plan of the experiment consisted of growing tomato plants in sand culture with complete nutrient solutions of different total salt concentrations; recording the resulting growth responses and making such determinations which seemed significant with respect to the relation between the concentration of the nutrient solution and the growth of the plant and to the development of blossom-end rot of the fruit.

Seeds of the progeny of a single Marglobe tomato plant were germinated in sand beginning July 3, 1934. The Marglobe variety was used, not only because of its great commercial importance but also because this variety is known to be relatively less susceptible to the development of blossom-end rot than many other varieties, particularly Stone, Dwarf Stone, and Baltimore (**29, 35**). It was thought, therefore, that any effect of the concentration of the solution upon the incidence of blossom-end rot in this variety would probably be more marked with many other varieties. Forty-eight carefully selected seedlings were transplanted on July 24 into thoroughly washed quartz sand in 2-gallon glazed crocks commercially known as coffee urn linings, one plant per crock. Two somewhat different grades of sand were used for comparison. One grade of sand was of medium fineness, from which all particles larger than 14-mesh and most all particles smaller than

TABLE I

MECHANICAL ANALYSIS OF TWO GRADES OF SAND USED, GIVING PERCENTAGE OF DIFFERENT SIZED PARTICLES

PARTICLE SIZE	SAND NO. 1	SAND NO. 2
	%	%
Larger than 2 mm.	0.0	2.3
2 mm. to 1 mm.	35.0	41.7
1 mm. to 0.5 mm.	63.7	53.3
Smaller than 0.5 mm.	1.3	2.7

40-mesh to the inch had been removed by screening; the other was a slightly coarser sand used without screening. The distribution of the various sized particles in each grade used is given in table I. All plants were initially supplied with the same complete nutrient solution of approximately one-quarter atmosphere osmotic value until August 14, when the plants had become well established. They then averaged 20 cm. in height, with a stem diameter of 5–6 mm., and had an average of 5 to 6 leaves each. At this time, six different nutrient treatments comprising six series with eight plants in each series were initiated, using complete nutrient solutions of five different salt concentrations. The cultures were replicated and so situated in the greenhouse as to eliminate, as far as possible between series, the effects of differences in the environmental factors of light, humidity, temperature, and air movement on the growth of the plants. These series will be designated A to F inclusive. All cultures in series A and B received the same solution of lowest concentration, namely, 0.08 atmosphere osmotic value, but at different rates of supply. Cultures in series C to F inclusive, received solutions of 0.44, 0.83, 1.7, and 3.1 atmospheres osmotic value, respectively. The rate of nutrient solution supply was approximately one liter per plant per day in all series except series A, which was four liters daily. This higher rate was employed to reduce to a minimum any nutrient deficiency which with the solution of this low salt concentration might limit growth if supplied at the lower rate as in series B. Table II gives the partial volume molecular concentrations and the parts per million of the salts, together with the osmotic and pH values of the solutions used in each series. The nutrient solutions were applied to the sand in each culture continuously by the method of SHIVE and STAHL (**23**). The relative proportion of the salts, which was the same in all the solutions except the lowest in concentration, had previously been found satisfactory for the growth of the tomato plant (**4**). Both nitrate and ammonium nitrogen were used in these solutions in order to reduce to a minimum the change in pH value of the solution which takes place during growth due to differential ion absorption.

TABLE II

NUTRIENT SOLUTIONS USED IN SERIES A TO F

NUTRIENT SOLUTION		NUTRIENT SALTS				
SERIES	OSMOTIC VALUE	TOTAL	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	$(NH_4)_2SO_4$
	atm.	*p.p.m.*	*Partial volume molecular concentrations*			
A & B	0.08	190	0.00026	0.00037	0.00050	0.00018
C	0.44	997	0.00106	0.00146	0.00356	0.00140
D	0.83	1,992	0.00211	0.00292	0.00711	0.00280
E	1.70	4,978	0.00528	0.00730	0.01775	0.00700
F	3.10	10,055	0.01055	0.01460	0.03550	0.01400

Each culture of a series was flushed daily with the solution used in that particular series to prevent salt accumulation. Daily records were taken of the maximum and minimum temperatures, the wet and dry bulb temperatures, and the evaporation from both black and white standardized spherical atmometer cups (**13**). The percentages of relative humidity were obtained from the wet and dry bulb thermometer readings with the aid of published tables (**17**). To aid good pollination, plants were jarred at appropriate times. On September 21, one-half of the plants in each series was harvested. Nutrient treatments were continued with those cultures remaining. The pH values of the nutrient solutions were obtained colorimetrically.

The osmotic values of the nutrient solutions and of the extracted plant juices were determined by means of the freezing point depressions of the liquids, with the Beckman thermometer according to the method of HARRIS and GORTNER (**9**). Appropriate corrections for undercooling were made. All reported osmotic values are those at 20° C. The tissue fluids used for the determination of osmotic values were obtained by extraction with a hand screw press from the representative tissue samples.

Determinations of refractive indices were made at controlled temperature with a Zeiss Abbe refractometer following the suggestions of GORTNER and HOFFMAN (**7**) with regard to plant tissue extracts. The values of water content of the tissues were obtained from SCHÖNROCK's table (**21**). The plants were harvested on the dates indicated and the tissues were rapidly dried in a current of air at about 70° C. The analytical fraction reported "leaves" included the petioles. The fraction reported "stems" included only that portion of the stem produced as new growth after the nutrient treatment in each of the several series was started. For the determination of osmotic values of tissue extracts, the fraction reported "stems" included both young stem and leaf tissues and the fraction designated "fruits" consisted of green, immature fruits from 2.5 to 3.5 cm. in diameter.

Experimental results

Environmental conditions

Daily records made of certain factors of the environment during the course of the experiment are given in figure 1. The lower curves in the diagram represent corrected values for the loss of water, in grams, by evaporation from the surface of the black and white Livingston standard spherical atmometer cups for each twenty-four-hour period. These values indicate wide differences in daily evaporating power of the air. For example, the daily rate of evaporation on September 12 was 36 gm. for the black cup, and 29 gm. for the white cup. Five days later, on September 17, the value for each cup was less than 4 gm. The days with very low evaporation rates were usually either very cloudy or rainy, accompanied by a somewhat lower temperature and high relative humidity. The days with high evaporation rates were usually those of high light intensity, accompanied by high temperatures and low relative humidity. Although no direct quantitative measure of light intensity itself was made, the differences between

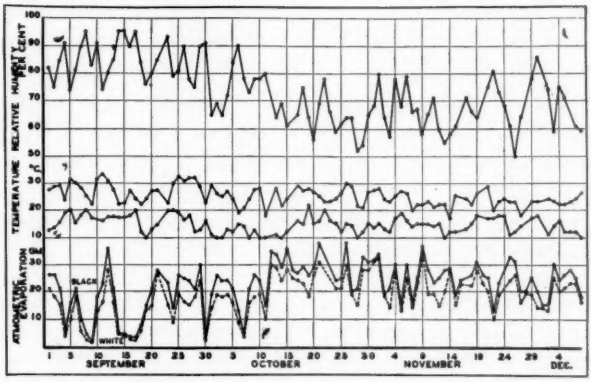

Fig. 1. Daily records of percentage relative humidity, maximum and minimum temperature, and rates of atmospheric evaporation from standard black and white atmometers.

the daily evaporation from the black and white atmometer cups reflect the relative amount of absorption of solar radiant energy, and vary directly with changes in light intensity (**12**). On bright days these differences were relatively large, for example, on September 12, September 25 to 29, October 1 to 4, October 8 to 10, and October 21 and 26, as indicated in figure 1. On dark days these differences were relatively small; for example, on September 8, 9, 14, 17, and 30, and October 7. Since the absorption of radiant energy greatly increases transpiration, as well as evaporation, the effect of light on transpiration has an important bearing upon the relation between the con-

centration of the solution and the occurrence of blossom-end rot of the fruit. Throughout the experiment there continued to be fluctuations from day to day in evaporation rates, but with the fall in the dewpoint and decrease in actual humidity of the outside air with the coming of cool weather, and with the artificial heating of the greenhouse in which this experiment was conducted, the average relative humidity in the greenhouse gradually decreased during October and November. Consequently, the average evaporation rates were higher than during September and early October.

The percentages of relative humidity were obtained from wet and dry bulb thermometer readings taken at 8 : 30 A. M. each day, and are represented on the upper curve in figure 1. Extreme air currents were not present in the greenhouse, and, therefore, the evaporation rates varied inversely with relative humidity in most cases. The values for percentage relative humidity, shown in figure 1, however, give a measure of the vapor pressure deficit at the time of the reading, whereas the values of evaporation rates given, are twenty-four hour rates. Therefore, while sudden changes in temperatures and absolute humidity taking place during the day are not reflected in the values for percentage relative humidity given in figure 1, nevertheless the results of such changes are integrated in the values for the twenty-four-hour rates of evaporation. The curve indicating relative humidity shows the wide fluctuation in values within periods of a few days duration; for example, from 91 per cent. on September 30 to 65 per cent. on October 1, and again from 78 per cent. on October 22 to 25 per cent. on October 28.

Although with a given amount of water vapor in the air, the percentage relative humidity is closely dependent upon the temperature, yet, the two curves in figure 1 giving maximum and minimum temperatures for the same twenty-four-hour period over which the daily evaporation rates were measured, show that there was considerably greater variation in evaporation rates and percentage relative humidity than there was in temperature. This was due to differences in rate of supply of water vapor to the air with any given increase in temperature, whether the water vapor came from air outside the greenhouse or from the soil surface in the greenhouse. A sudden increase in temperature, with a low rate of water vapor supply to the air from the surroundings, was accompanied by a sharp drop in percentage of relative humidity and a rise in the evaporating power of the air. A slow increase in temperature, accompanied by a high rate of water vapor supply from the surroundings, was accompanied by little change in the relative humidity with increase in temperature. Therefore, as far as the relation between the concentration of the nutrient solution to the occurrence of blossom-end rot may be dependent upon the percentage of relative humidity or the evaporating power of the air, so far must temperature remain an *indirect* factor.

GROWTH AND DRY WEIGHT PRODUCTION OF TOPS

The tomato plants in each of the several series started to grow rapidly, but differences in nutrient supply were soon reflected in the growth responses. Representative plants in each of the several series, are shown in figure 2 as they appeared on September 4, three weeks after the beginning of the nutrient treatments. Plants in series A, C, D, and E, with which the

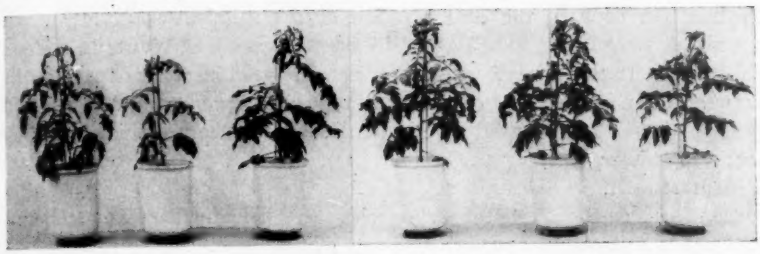

FIG. 2. Tomato plants on September 4, grown with complete nutrient solutions of different osmotic concentrations. Left to right: series A, 0.08 atm.; series B, 0.08 atm.; series C, 0.44 atm.; series D, 0.83 atm.; series E, 1.70 atm.; series F, 3.10 atm. Nutrient solution of series A supplied at a rate four times that in series B.

nutrient solutions of low and intermediate concentrations were used, started and continued to make relatively more rapid growth than plants in either series B or F. The leaves in the plants of the former series were darker green and larger in size, and the mesophyll of the leaves more savoyed, the plants taller, the stems thicker with more axial sprouts, than was the case of the plants in series B, with the solution of lowest concentration, or in series F, with the solution of highest concentration.

A small amount of injury to the tips of a few leaves of some plants grown with the solutions of the intermediate and higher concentrations appeared following extended periods of cloudy weather. This injury to tissues was not typical wilting, although there was a slight resemblance, but was associated with the fairly high concentrations of ammonium nitrogen in the solutions in these series and its accumulation in these tissues during periods when the assimilation rate of this ion was low (**31**). Aside from this injury, no other type of specific tissue injury was noted in the plants grown with solutions of high concentrations throughout the experiment.

No significant growth differences associated with the different grades of sand used occurred, and, therefore, further comparisons between series will be made only with reference to the nutrient treatments in the different series.

The differences in growth of the plants in the different series were apparent throughout the experiment. At the time of harvest on September 21 the plants in series A were larger than those in series B and even slightly

greater than those in series C in spite of the fact that plants in series C were grown with a solution of an osmotic concentration more than four times as great, but supplied at one-fourth the rate as the solution in series A. The relatively poor growth of plants in series B was due to a lack of available nutrients, particularly nitrogen, and shows, as has been pointed out by HOAGLAND (10) and JOHNSTON and HOAGLAND (11), that the rate of renewal of solution as well as the initial concentration are both important factors in the relation between the concentration of the nutrient solution and the growth of the plants. The less rapid rate of growth of plants in series F compared with that in series C, D, and E, considered in terms of the relation between the concentration of the solution and nitrogen assimilation of the plants, will not be discussed here in detail. Reference has previously been made to this relation by the writer (20), and also by NIGHTINGALE and FARNHAM (18).

One-half of the plants of each series were harvested on September 21 and figure 3 shows representative plants at this time. Table III gives the

FIG. 3. Tomato plants on September 21, grown with complete nutrient solutions of different osmotic concentrations. Left to right: series A, 0.08 atm.; series B, 0.08 atm.; series C, 0.44 atm.; series D, 0.83 atm.; series E, 1.70 atm.; series F, 3.10 atm. Nutrient solution of series A supplied at a rate four times that in series B.

values for the green weight, dry weight, and the percentage dry weight of various parts of these plants. There was little difference between the total green weights per plant in series C, D, and E—the values being 256, 260.1 and 259.7 gm. respectively. The green weight of series A plants, 228.6 gm., was somewhat lower than these, while the green weight per plant in series B, 122.2 gm., was less than one-half this value. The green weight per plant in series F was 184.2 gm. In general, the dry weights per plant correspond relatively in order of value with the green weights in the respective series. The total dry weight per plant of series E, however, was 29.86 gm., which

TABLE III

AVERAGE GREEN WEIGHT, PERCENTAGE DRY WEIGHT, AND TOTAL AVERAGE DRY WEIGHT PER PLANT OF LEAVES, STEMS, AND FRUITS OF TOMATO PLANTS GROWN WITH SOLUTIONS OF DIFFERENT OSMOTIC CONCENTRATIONS

SERIES	OSMOTIC CONC.	AVERAGE GREEN WEIGHT PER PLANT				PERCENTAGE DRY WEIGHT			TOTAL AVERAGE DRY WEIGHT PER PLANT			
		LEAVES	STEMS	FRUITS	TOTAL	LEAVES	STEMS	FRUITS	LEAVES	STEMS	FRUITS	TOTAL
	atm.	*gm.*	*gm.*	*gm.*	*gm.*	%	%	%	*gm.*	*gm.*	*gm.*	*gm.*
A	0.08*	130.6	48.3	49.7	228.6	11.43	10.85	7.40	14.93	5.26	3.68	23.87
B	0.08	58.5	23.7	40.0	122.2	11.70	10.85	6.73	6.84	2.57	2.69	12.10
C	0.44	160.5	53.4	42.1	256.0	11.27	10.06	8.25	18.09	5.37	3.47	26.93
D	0.83	172.1	59.6	28.4	260.1	10.90	9.41	7.85	18.95	5.61	1.93	26.49
E	1.70	170.8	66.8	22.1	259.7	12.16	10.58	9.14	20.77	7.07	2.02	29.86
F	3.10	115.5	42.0	26.7	184.2	13.00	12.12	10.24	15.02	5.09	2.73	22.84

* Nutrient solution supplied at a rate four times that in series B.

was greater than the average dry weight of the plants in any of the other series. At this phase of plant development, the fruits on the plants on all series were small and immature, and although the green and dry weights of fruits in series D, E, and F were less than those of series A, B, and C, these differences were reversed as fruit maturity progressed.

The tissues of plants in series F were lower in water content than corresponding tissues in the plants of other series as shown by the percentages of dry weight. For example, the percentage dry weight of the stems of series F was 12.12 as compared with 9.41 for series D, which indicates that growth of the plants in series F was limited by an available water supply for the various tissues, and that the water content of the tissues of these plants decreased with increase in salt concentration of the nutrient solution.

Differences in the water content of ripe, mature fruits grown in the several series were much greater than the differences in the water content of the immature fruits. Table IV gives the percentage of dry weights of

TABLE IV

PERCENTAGE DRY WEIGHT OF GREEN IMMATURE AND RIPE FRUITS HARVESTED ON DECEMBER 4 FROM TOMATO PLANTS GROWN WITH COMPLETE NUTRIENT SOLUTIONS OF DIFFERENT OSMOTIC CONCENTRATIONS

SERIES	OSMOTIC CONC.	FRUITS	
		PERCENTAGE DRY WEIGHT	
		GREEN	RIPE
	atm.	%	%
A	0.08*	.....	4.54
B	0.08	.....	4.71
C	0.44	7.12	6.69
D	0.83	6.45	7.11
E	1.70	7.58	8.44
F	3.10	10.65	.....

* Nutrient solution supplied at a rate four times that of series B.

green fruits and of ripe fruits harvested on December 4 from each series. Only normal fruits with no blossom-end rot are here represented. Where no values appear in the table, fruits of that series were unavailable. The percentage dry weights of ripe fruits of series A and B were 4.54 and 4.71 respectively, whereas the percentage dry weight of ripe fruits in series E was 8.44, or nearly twice as great, and reflects the great difference in the availability of water supply for these respective tissues.

These values of green and dry weight production show that in sand culture with nutrient solutions of 0.44, 0.83 and 1.7 atmospheres, tomato plants made excellent growth; that with a nutrient solution having an osmotic value of 0.08 atmospheres, good growth resulted only when the nutrient solution was supplied at a rate sufficiently rapid to furnish an adequate nutrient supply and that although good growth resulted with a nutrient solution of 3.1 atmospheres osmotic value, growth of these plants was limited by factors other than mere quantitative nutrient supply. The relation between the water content of the tissues and the osmotic value of the extracted juices will be discussed later.

Root development

The roots of the plants in the several series presented distinct differences in appearance. The roots of the plants grown with the solution of highest concentration were more slender and much more wiry than the roots of the plants in the other series. When bent double, these roots did not break readily as did those of the plants grown with the solution of the lowest concentration. The roots apparently had matured early before much increase in diameter had taken place. Many of these roots in the series F plants were light brown in color, except toward the tips which were white, indicating early suberization of root tissues. The individual roots of the series F plants were not as long as those of the series A and B plants. The roots of series A and B plants were brittle, and usually broke when bent double. They were a uniform light yellow to white in color, and were maturing relatively slowly. Each root was larger in diameter and longer than those of series F, giving the root system a coarser and more extensive appearance. The roots grown with the solution of intermediate concentrations, namely, series C, D, and E, were intermediate, in condition and appearance, between the roots of the plants grown with the solutions of highest and lowest concentration. Roots of series C and D plants were excellent, and typical of roots produced under optimum sand culture conditions.

The differences in root structure noted in the several series offer an additional explanation for the observed decrease in water absorption and water requirements of plants grown in solutions of high salt concentration. When the roots mature rapidly with early suberization of epidermal cells, the proportion of active absorbing surface to total root surface is relatively low, and the rate of water absorption, by tissue which has thus matured, is minimized. The differences in roots of these plants grown with solutions of different concentration were similar to the differences in the roots of sweet pea which have been described by Nightingale and Farnham (**18**).

The possible importance in the several series of the effects of certain ions, due to their specific nature, and aside from their colligative osmotic proper-

ties—for example, calcium and phosphate, on the rate of maturity of and the absorption of water by the root tissues—is not to be here disregarded, but has not been completely evaluated in this experiment.

Turgor and guttation

Soon after the beginning of this experiment, there was apparent a difference in the internal water relations of the plants of the several series. Although there was no pronounced and typical wilting of the leaves of any of the plants during the course of the experiment, differences in turgor of the tissues were evident. The mesophyll cells in the leaves of plants in series A, B, C, and D, grown with the solutions of lower concentrations, were glistening and shiny in appearance, which is typical of cells with high turgor. Similar leaf tissue cells in series E and F lacked somewhat this glistening, shiny appearance. The leaves of series E and F with the solution of highest concentration did not have as high turgor as did those of the other series. The younger leaves of the plants in these latter series, during the middle of the day when the evaporation rate was highest, dropped slightly more than at any other times of the day. The leaves on plants in series A to D appeared to have maximum turgor at all times of the day.

The appearance of guttation on the plants of some series, and not on others, gave further evidence of differences in turgor. Drops of guttated liquid occurred on the leaves of plants in series A and D during periods of very low transpiration, on September 3, 4, 7, 8, 10, and 14. As indicated in figure 1, the percentage relative humidity of the air was very high on these days, namely, between 85 and 95. Guttation was not observed on any of the plants in any of the series when the relative humidity was much below 85 per cent. and during the last two months of the experiment this value was recorded only twice.

Figure 4 shows a plant on August 25, when the relative humidity was 95 per cent., from series B with large drops of guttated liquid on the tips and margins of the leaves. At no time did guttation take place from the leaves of any plants grown in series E and F, with the solutions of high concentration.

There was considerable difference in the relative amount of guttation in the several series. Practically all the leaves of plants in series A and B showed copious guttation. Guttation was not so abundant from the leaves on series C plants, although most leaves of the plant showed some. On series D, however, under the same conditions, only the younger leaves showed this phenomenon. Therefore, under conditions of high humidity where transpiration rates were low, positive internal pressures, resulting in guttation, occurred with the solutions of the lower concentration used. With solutions of the higher concentrations, however, such great positive pressures did not

occur. Likewise in the same plant turgor differences made evident by differential guttation were maintained in the cells of the respective tissues by differences in either osmotic or imbibitional forces, or a combination of both. The importance of the maintenance of such differences in pressure of the hydrostatic system of different tissues to the incidence of blossom-end rot of the fruits will be discussed later.

FIG. 4. Tomato plant grown with nutrient solution of low osmotic concentration (0.08 atm.) showing drops of guttated liquid on leaves.

FRUIT DEVELOPMENT AND INCIDENCE OF BLOSSOM-END ROT

The most striking external differences in the growth of plants in the several series were in the development of fruits, and the incidence of blossom-end rot. These differences were caused primarily by the same factor—namely, relative differences in the adequacy of the water supply for fruit development. The lower relative water requirements of plants grown with solutions of high concentrations than with low concentrations is known (**16, 22**). Plants in all series produced fertile blossoms which set fruit freely. Figure 2 shows the plants as they came into bloom. Fruits set slightly earlier on plants with the solutions of low concentration as may be seen in table V, but at the time of the first harvest on September 21, plants in all series bore small fruits. Individual fruits in all series developed at approximately the same rate until they reached a size of from 2.5 to 3.5 cm. in diameter. Up to this phase of development, as shown in figure 3, blossom-end rot did not occur in any of the series, with two exceptions, but from this

TABLE V

NUMBER OF FRUITS SET PER PLANT IN EACH SERIES OF CULTURES GROWN WITH NUTRIENT SOLUTIONS OF DIFFERENT OSMOTIC CONCENTRATIONS ON THREE DATES DURING EARLY PART OF EXPERIMENT

SERIES	OSMOTIC CONC.	FRUITS SET PER PLANT		
		SEPTEMBER		
		13	17	21
	atm.			
A	0.08*	1.0	2.4	3.0
B	0.08	0.6	2.0	2.3
C	0.44	1.1	2.6	3.3
D	0.83	1.0	2.0	2.3
E	1.70	0.5	2.9	4.8
F	3.10	0.4	3.0	4.3

* Nutrient solution supplied at a rate four times that of series B.

point on fruits in series E and F increased in size much more slowly, and under favoring conditions developed blossom-end rot much more rapidly than did the fruits in other series. Figure 5 shows fruits chosen on October 14 at a somewhat similar size, namely, 4.7 to 5.7 cm. When environmental

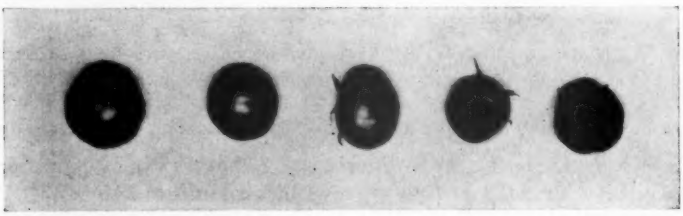

FIG. 5. Immature fruits on October 14, grown with complete nutrient solutions of different osmotic concentrations. Left to right: series A, 0.08 atm.; series C, 0.44 atm.; series D, 0.83 atm.; series E, 1.70 atm.; series F, 3.10 atm. Fruits from series E and F exhibit blossom-end rot.

conditions favored the development of blossom-end rot, the initial symptoms of this disease appeared more quickly on fruits of this size and phase of maturity, than on either the smaller less mature, or on the larger more mature fruits. Maturity, or the character and composition of the fruits, rather than the size itself, is the important factor in this relation, and this will be discussed in connection with the ability of the affected tissues to obtain and retain an adequate water supply for tissue development.

The difference in fruit size of the several series continued to be greater until the fruits ripened. Table VI shows the average weight of individual ripe fruits in the several series on several harvest dates, for both normal fruits and those exhibiting blossom-end rot injury. The average weight

TABLE VI

AVERAGE WEIGHT PER RIPE FRUIT ON EACH OF THREE HARVEST DATES, TOTAL FINAL AVERAGE WEIGHT PER FRUIT, TOTAL NUMBER OF FRUITS PER PLANT, AND PERCENTAGES OF TOTAL NUMBER OF FRUITS WITH BLOSSOM-END ROT FROM PLANTS GROWN WITH NUTRIENT SOLUTIONS OF DIFFERENT OSMOTIC CONCENTRATION

NUTRIENT TREATMENT		AVERAGE WEIGHT PER FRUIT			SUMMARY		
SERIES	OSMOTIC CONC.	Nov. 4	Nov. 19	Dec. 4	TOTAL FRUITS PER PLANT	AV. WT. PER FRUIT	PERCENTAGE OF TOTAL FRUITS
			Normal fruits				
	atm.	*gm.*	*gm.*	*gm.*		*gm.*	%
A	0.08*	140	129	111	5.5	127	100
B	0.08	.....	87	51	3.0	69	100
C	0.44	105	82	79	2.5	89	83
D	0.83	114	117	44	3.8	92	72
E	1.70	.....	38	47	3.3	43	23
F	3.10	.....	25	10	0.5	18	20
			Fruits with blossom-end rot				
A	0.08*	.....	.....	.....	0.0	.....	0
B	0.08	.....	.....	.....	0.0	.....	0
C	0.44	27	44	.....	0.5	36	17
D	0.83	91	.....	70	1.3	81	28
E	1.70	54	.....	57	2.5	56	77
F	3.10	26	58	31	2.0	38	80

* Nutrient solution supplied at a rate four times that in series B.

per ripe normal fruit varied from 127 gm. in series A, to 18 gm. in series F. The small size of the few fruits in series F, which ripened normally, was partly due to imperfect fruit development caused by an occasional incomplete pollination of the original blossoms. Fruits in series F, which developed from completely pollinated blossoms, were sowewhere larger and averaged 38 gm. in weight when ripe, but these fruits invariably developed blossom-end rot. Figure 6 shows the fruits on a plant from series A, on November 4. Although the ripe fruits harvested from plants in this series,

FIG. 6. Representative tomato plant from series A, grown with complete nutrient solution of 0.08 atm. osmotic value. The ripe fruits are of large size and show no blossom-end rot.

at this time, averaged 140 gm., the largest fruits weighing less than 200 gm., yet, one of these fruits, shown on the lower cluster in figure 6, when harvested later weighed 400 gm., an unusual size for a fruit of this variety. Figure 7 illustrates typical fruits from each series as they appeared when ripe. The fruit from series A on the left was 7.4 cm. in diameter; that from series F, showing blossom-end rot, was 3.6 cm. in diameter. The two fruits on the

right show the typically shrunken appearance of ripe fruits with blossom-end rot. The fruits in series B, and to a slight extent in series A, showed, as did the growth of the plants themselves, the effect of a somewhat limited nitrogen supply. These latter fruits were somewhat more irregular in outline and less globular in shape than fruits in series C and D, and the flesh of the fruit around the stem of many showed a slightly yellow tinge of color as the fruits ripened.

As shown in table VI, blossom-end rot occurred on most fruits grown with solutions of high concentration and did not occur on solutions of the lowest concentration used. The percentage of total fruits with blossom-end rot was: series F, 80 per cent.; series E, 77 per cent.; series D, 28 per cent.; and series C, 17 per cent. In series A and B there was no blossom-end rot. The only fruits in series F which failed to develop blossom-end rot were the occasional very small fruits averaging 18 gm. in weight, which, as was previously

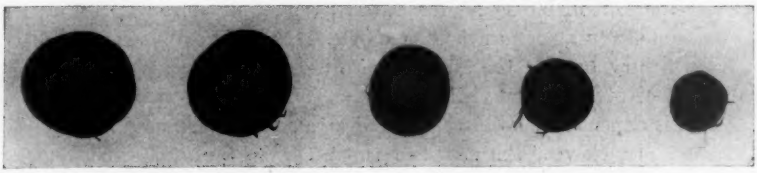

FIG. 7. Ripe fruits on November 4, grown with complete nutrient solutions of different osmotic concentrations. Left to right: series A, 0.08 atm.; series C, 0.44 atm.; series D, 0.83 atm.; series E, 1.70 atm.; series F, 3.10 atm. Fruit from series E and F show blossom-end rot in advanced stages.

indicated, never reached the size when blossom-end rot was found to most readily develop.

The initial symptoms of blossom-end rot appeared most often during periods of bright, warm weather, accompanied by high evaporation rates, which followed periods of cool, dark weather accompanied by low evaporation rates. The initial symptoms were externally apparent in a very few days after such a sudden change in these environmental factors. The differences in evaporation rates of the air by their direct effect upon the water relations of the plant appeared to be the principal factor influencing the development of blossom-end rot, rather than the indirect factors of light and temperature. Such changes in evaporation rates occurred frequently during the experiment. Figure 5 shows green fruits in series E and F with blossom-end rot as they appeared on October 14, which was the third day of an extended period of relatively high evaporation rates (fig. 1) following two periods of low evaporation rates,—one on October 7 and one on October 11.

After blossom-end rot had seriously involved a considerable portion of the tissues, fruits rarely ripened normally thereafter. When conditions

associated with the occurrence of blossom-end rot recurred repeatedly, the affected part of the fruit increased in size and involved a large portion of the fruit. Figure 8 shows two stages in the advance of this disease to a point

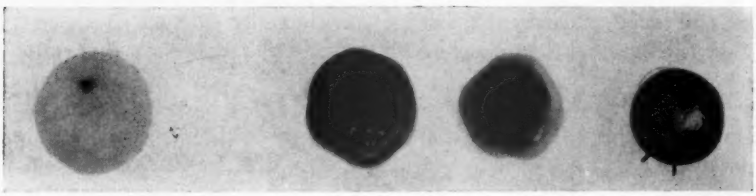

FIG. 8. Tomato fruits grown in sand culture. Left, normal fruit from series A; right, three fruits from series F showing blossom-end rot in several stages of development.

where recovery did not take place. When, however, the conditions favoring the development of blossom-end rot, did not reoccur before fruit maturity, an occasional fruit, such as the one on the right in figure 8, ripened with no further injury beyond the slight browning of the outer epidermal tissue which constituted the first visible symptoms of the occurrence of this disease.

In this experiment both nitrate and ammonium were used as sources of nitrogen. In view of the repeated references to the greater incidence of blossom-end rot where ammonium nitrogen was used, than where nitrate nitrogen was used, it may be wondered whether the form of nitrogen supplied in the nutrient solution was an important direct factor associated with the development of this disease. Comparisons of the effect of the two forms of nitrogen on the rate of incidence of blossom-end rot were not made in this experiment. But in preliminary experiments, under almost the identical cultural, seasonal, and environmental conditions which obtained during a part of this experiment, and where the nutrient solutions contained nitrate as the sole source of nitrogen, almost all of the fruits developed blossom-end rot when the osmotic concentration of the solution used was 1.75 atmospheres. When the osmotic value was decreased to one atmosphere, keeping the proportion of the salts the same, not a single fruit developed blossom-end rot thereafter. The development of blossom-end rot is not necessarily to be associated solely with either ammonium or nitrate nitrogen. Salts containing either form of nitrogen if present in high concentrations obviously exert an osmotic effect upon the absorption of water. However, aside from the osmotic effect concerned, if the use of nitrogen in high concentration in any form which can easily be assimilated results in an extremely vegetative growth of a tomato plant, such a plant will have a small proportionate root system, a low proportionate amount of water conducting tissue, thin cell

walls, and other features (19) which, under conditions of high evaporation, make it wilt more quickly than a plant grown with a relatively low rate of nitrogen assimilation. Fruits on such a plant are relatively more susceptible to the development of blossom-end rot than are those of the low nitrogen plant. The great relative rate of incidence of blossom-end rot of the fruits of vigorously vegetative plants is a matter of common observation and has been referred to.

Osmotic values of expressed tissue juices and water supply of fruits

In order to account for the loss of water from the fruits which occurs during the development of blossom-end rot, determination of the osmotic values of expressed tissue juices of fruits and young stems and leaves of the plants in the several series were made. Table VII gives these values. The osmotic values of the fruit tissues ranged from 6.68 atmospheres in series A and C to 10.79 atmospheres in series F, and for the young stem and leaf tissue from 8.19 atmospheres in series B to 12.99 atmospheres in series F. There is thus a difference of over 4 atmospheres between the osmotic values of the expressed juices of analogous tissues of plants grown with solutions of the lowest and highest concentrations. These differences in osmotic values show that there exist great differences in the availability of water in the tissues of these plants in the several series. There were also differences in the actual water content of the stem tissue extract in the several series, as determined by the refractometer, ranging from 94.55 per cent. for series A to 92.55 per cent. for series F. The small differences in water content, compared with the total percentage assume a greater significance when the percentage of total solids, which include solids of all degree of osmotic activity, are compared. These values vary from 5.45 per cent. in the case of series A to 7.45 per cent. in series F, representing a difference of 37 per cent. over the series A value. The relative *osmotic* values of the fruit and stem tissue extracts in the several series, taking the respective values in series A as 100, are also given in table VII. The relative osmotic values of the series F plants are 162 for the fruit and 155 for the stem. In the cases of series D, E, and F, at least, it may be seen that the relative osmotic values of the fruits and of the stems are higher than the relative values for the percentage of total solids of the stems. This shows that there was a higher proportion of osmotically active solids in the tissues of the plants in these three series and emphasizes the probable importance of the inorganic salts in the osmotic relations of these tissues.

In each series the osmotic values of the extracts of the stem and leaf tissue exceeded those of the fruit tissue. These differences ranged in magnitude from 1.62 atmospheres in series A to 3.63 atmospheres in series C, and indi-

TABLE VII

OSMOTIC VALUES OF TISSUE EXTRACTS OF SMALL GREEN FRUITS AND YOUNG STEMS, AND PERCENTAGE OF WATER AND SOLIDS OF THE EXTRACT OF STEMS OF TOMATO PLANTS GROWN WITH COMPLETE NUTRIENT SOLUTIONS OF DIFFERENT OSMOTIC CONCENTRATIONS

Nutrient treatment		Tissue extract							
		Osmotic values					Percentage of water	Percentage of solids	Relative values Series A = 100
Series	Osmotic conc.	Fruit	Stem	Relative values Series A = 100		Gradient between fruit and stem	Stem	Stem	Stem
				Fruit	Stem				
	atm.	*atm.*	*atm.*			*atm.*	%	%	%
A	0.08*	6.68	8.30	100	100	1.62	94.55	5.45	100
B	0.08	.....	8.19	.....	99	.....	94.40	5.60	103
C	0.44	6.68	10.31	100	124	3.63	94.25	5.75	107
D	0.83	7.71	10.51	115	127	2.80	94.00	6.00	110
E	1.70	9.17	12.21	137	147	3.04	93.85	6.15	113
F	3.10	10.79	12.99	162	155	2.20	92.55	7.45	137

* Nutrient solution supplied at a rate four times that in series B.

cate that a water deficit must surely exist in the tissues of the fruit and that under conditions of high transpiration rates, not only did a continued water supply become unavailable to the tissues of the fruit, but actual withdrawal of water took place from these tissues. In plants in series E and F particularly, this loss of water from the tissues of the fruits was great enough to bring about the tissue desiccation and cell collapse known as blossom-end rot.

These results are in agreement with those of HAAS and KLOTZ (**8**), who report higher osmotic values for the extracted sap of lemon leaves than of the fruits with attendant loss of water from the fruits; with those of BARTHOLOMEW (**2**), who by dendrographic measurements found actual water loss from lemon fruits; and with MACDOUGAL (**14**), who by similar measurements reported loss of water from tomato fruits.

It has been assumed that during the development of blossom-end rot the principal loss of water from the fruits took place through the pedicel, rather than from the surface of the fruit. This apparently was true since fruits of series F in a state of maturity, such that blossom-end rot developed rapidly under favoring conditions if allowed to remain attached to the plant, lost water much less rapidly after removal from the plant than when still attached, and after removal from the plant did not develop blossom-end rot. Transpirational loss, therefore, from the fruit surface was concluded to be negligible compared with the loss through the pedicel to the stems and leaves.

Some explanation must account for the fact that severe blossom-end rot developed in fruits of series F plants when the determined osmotic gradient between fruits and stems was found to be 2.20 atmospheres whereas this disease did not develop in fruits in series A plants when the osmotic gradient between fruits and stems was 1.62 atmospheres, only slightly less. The osmotic gradients between fruits and stem tissue in the plants of the other series was even greater than either of these two differences. It is significant that the smallest gradient occurred in series A plants where blossom-end rot did not develop; and this alone may primarily account for the phenomenon. However, an additional explanation seems pertinent. The existence of an osmotic gradient between two tissues does not *per se* necessarily imply movement of water. The difference in osmotic pressure, which might otherwise be expected to result in water movement, can be counteracted by imbibitional forces establishing a gradient in the opposite direction. No movement of water then takes place and the tissues retain their supply against a determined osmotic gradient.

This relation between the osmotic and imbibitional forces of fruit tissue on the one hand, and leaf and stem tissue on the other hand, apparently obtained in series A and B. Moreover, accompanying a considerable variation of osmotic concentration of cell sap which is known to occur periodically (**32**), cells, although losing a slight amount of water at certain times of the

day, may yet recover without serious injury when water is again available, provided loss of water does not continue to take place too long. Any loss of water from fruits of series A and B plants, which may have occurred, was undoubtedly very slight and of short duration owing to the low resistance to water absorption which the solution of low concentration used with these series offered. Loss of water from the tissues of fruits in series C, D, E, and F, on the other hand, took place not only more rapidly as indicated by the greater osmotic gradients, but undoubtedly this loss of water occurred over a longer period of time owing to the higher resistance to water absorption which these solutions offered as compared with the nutrient solution used with series A and B.

The notably high imbibitional forces of young cells (**5, 24, 25, 26**), often exceeding in value the osmotic forces, undoubtedly accounts for the fact that very young, actively growing tomato fruits did not develop blossom-end rot even under the conditions of high transpiration intensities, and the severe fluctuations in evaporation rates which occurred at certain times during this experiment.

During the development of blossom-end rot there is, in addition to a loss of water from the fruit as a whole, competition for water between different tissues of the same fruit. By the refractometer method, it was found that differences in water content of the extracts of various tissues of the same fruit did exist. The water content of the wall tissue near the stem end of the fruit was 93.05 per cent. whereas the water content of the tissue of the wall near the blossom-end of the fruit was 93.6 per cent. The water content of the juice of the seed cavity was still higher – 93.85 per cent. Although differences in water contents do not necessarily prove that attendant differences in osmotic values exist, yet, the close inverse relation between water content and osmotic value of plant tissues is very common as STODDART (**27**) has shown.

GAIN OF WATER BY FRUITS

Whereas, associated with the development of blossom-end rot, tomato fruits on plants grown with nutrient solutions of high concentration lost water during periods of high transpiration, under the reverse environmental conditions, when grown with solutions of low concentration and under low transpiration, fruits gained water. The relatively high water content of the fruits in series A and B has been noted. The gain in water by fruits following sudden change of environmental conditions may be so rapid that actual tissue rupture takes place. Such cracking is very common in some varieties of tomatoes in the field (**6**), when they are almost ripe, particularly during a rain which follows a period of bright warm weather. Such cracking or splitting of the fruits usually occurs coincidentally with guttation from the

leaves, under conditions of very low transpiration. In this experiment, although there was an occasional fruit in series A which showed this splitting, environmental conditions were not particularly favorable to the occurrence of this cracking during the latter part of the experiment when the fruits were ripening. However, such splitting of nearly ripe fruits has occurred at other times repeatedly with solutions of low concentration during a cloudy period of very high humidity immediately following a period of bright weather. There was no splitting whatever of the fruits in series C, D, E, or F. Furthermore, whereas blossom-end rot develops principally on green fruits, cracking occurs largely with ripe or nearly ripe fruits which are high in sugars.

A mechanism of tissue rupture in Stayman apples, which appears to be similar to that involved in cracking of tomato fruits, has been reported by VERNER (**34**) to occur under a combination of environmental conditions just the reverse of those which, in this experiment, were found to be associated with the development of blossom-end rot of tomato fruit. Cracking of the external tissue of the apple occurred when underlying and adjacent fruit tissue had a high osmotic value with reference to the surrounding tissue, but this cracking took place only under conditions of high humidity. Under these conditions of low relative transpiration, water in the plant became more available and moved into the tissues concerned. This reported injury to the apple tissue occurred usually during or following a rain and therefore there undoubtedly was a coincidental decrease in the salt concentration of the soil solution, although these values were not reported. As VERNER pointed out, however, the incidence of the injury was not solely correlated with any particular water content of the soil.

MODIFYING FACTORS

Factors of the environment which more or less directly affect the water supply of the diseased tissue of the tomato fruit may modify the relation between the concentration of the nutrient solution and the development of blossom-end rot.

First, the rate of change of nutrient solution is an important factor. A rapid movement and abundant supply of solution in the substrate tends both to minimize the rate of change in salt concentration of the absorbing film of the roots and also to keep to a minimum the attractive force of the substrate particles for water—both factors of which affect the absorption of water by the roots. In this experiment the rate of nutrient solution supply was the same in all the series except series A, where the rate of supply was four times that of the other series. Although two slightly different sized sand particles in the substrate were used, there was no apparent difference in the incidence or rate of development of blossom-end rot of the fruits grown with the grades

of sand used. There was undoubtedly in the cultures of each series some variation in the concentration of the absorbing films and in the attractive force of the particles of the substrate for water under the varying rates of transpiration, but such changes are not considered the principal limiting factors in these experiments.

Secondly, since the pH value of the nutrient solution affects directly or indirectly the absorption of water by the plant, this factor may also become important in the relation between the concentration of the solution and the incidence and development of blossom-end rot of the tomato fruit. The original pH values of the different nutrient solutions herein employed were largely dependent upon the concentration of the phosphate ion, reference to which, with regard to the growth of roots, has already been made. Table VIII gives the pH values of the solutions in each series before and after

TABLE VIII

AVERAGE pH VALUES OF EXCESS SOLUTIONS ESCAPING FROM THE CULTURES IN EACH SERIES RECEIVING NUTRIENT SOLUTIONS OF DIFFERENT OSMOTIC CONCENTRATIONS, AS COMPARED WITH INITIAL pH VALUES OF EACH SOLUTION

SERIES	OSMOTIC CONC.	pH VALUE OF NUTRIENT SOLUTIONS			
		INITIAL	FINAL		
			SEPT. 2	SEPT. 14	NOV. 21
	atm.	*pH*	*pH*	*pH*	*pH*
A	0.08	5.2	5.3	5.5	4.2
B	0.08	5.2	5.4	5.7	4.0
C	0.44	5.0	5.2	5.2	4.1
D	0.83	4.9	4.9	5.1	4.1
E	1.70	4.8	4.4	4.5	4.1
F	3.10	4.7	4.4	4.1	4.1

passing through the cultures. Previous to the time of the first harvest on September 21, the pH values of the escaping solutions in series A, B, and C were each slightly higher than they were initially in the respective series. The pH values of the solutions escaping from the cultures in series E and F were lower than they were initially. During the latter part of the experiment, however, when distinct differences in the rate of incidence of blossom-end rot in the several series occurred, the solutions escaping from cultures in all series were approximately the same in pH value, namely, 4 to 4.2. Therefore, in this experiment observed differences in the pH value of the solutions in the several series are not in themselves considered principal limiting factors in the relation between the salt concentration of the nutrient solution and the incidence and rate of development of blossom-end rot.

Thirdly, the decrease in the rate of water absorption, which is associated with a low available oxygen supply to the roots (**16**) and the increase in suction pressure of the root cells attendant with low oxygen supply (**33**) may become a very important factor in affecting the development of blossom-end rot of the tomatoes grown in nutrient solutions. Thus, this disease of the fruit has been produced in this laboratory when the plants were grown in nutrient solutions of approximately only one-half atmosphere osmotic value but when insufficient aeration of the culture solution was provided.

The external factors which directly or indirectly affect transpiration, such as light intensity, the humidity, temperature, and rate of movement of the surrounding air, are likewise important factors in the development of blossom-end rot. The extent of regulation of transpiration by stomatal movement incident to changes in light intensity was not recorded in this experiment, but the importance of such regulation is not to be underestimated. The close association between changes in light intensity and the development of blossom-end rot in this experiment has, however, been previously noted, as well as the reported decrease in the rate of incidence of this disease on the fruits of shaded plants grown in soil compared with unshaded plants. The effect of the degree of humidity and temperature have been discussed, but the importance of the rate of air movement upon the development of blossom-end rot through the indirect effect upon the transpiration intensity should also be emphasized.

Before concluding the discussion of the relation between the solute concentration of the nutrient solution and the incidence of blossom-end rot of the fruit, the significance of wide variation in the magnitude of the saturation deficit of the tissues should be re-emphasized. Whereas, rapid growth of all tissues is coincident with relatively *low* saturation deficits, the occurrence of blossom-end rot must be coincident with *high* saturation deficits of the cells concerned. With culture solutions of high salt concentrations, the relatively high resistance to water absorption by the plants at a rate rapid enough to replace transpiration loss increased the magnitude of the saturation deficits of these plant tissues when the rate of transpiration was high, as compared with that present in these same tissues at other times when the transpiration rate was much lower. Thus, whereas water was available for the growth of fruits when the transpiration rates were relatively low, yet withdrawal of water from the affected tissues of the fruit took place when the transpiration rates were relatively high. Whether blossom-end rot would develop under conditions of constant low evaporation rates if the tomato plants were grown with solutions of high concentration, was not determined.

Conclusions

The close dependence of the plant upon an abundantly available water supply for the rapid growth of tissues and for the development of fruit is emphasized.

Rapid development of vegetative tissues and fruit of the tomato is facilitated with nutrient solutions of low concentration provided an abundant supply of the solution is available. With solutions of high salt concentrations, growth of the tissues may be limited by lack of available water.

A concomitant result of this lack of a readily available water supply for the tissues of the plant, is the development of blossom-end rot of the fruit which occurs to a high degree when nutrient solutions of high salt concentrations are employed.

Any factor which either seriously restricts the rate of absorption or greatly increases the rate of transpiration of water by the plant will increase the probability of the incidence and development of blossom-end rot of the fruit.

Summary

1. Tomato plants from a selected strain of the Marglobe variety were grown in sand culture in the greenhouse with nutrient solutions of five different osmotic concentrations; namely, 0.08, 0.44, 0.83, 1.7, and 3.1 atmospheres. A comparison was made of the effects on growth of two rates of supply of the solution of lowest concentration.

2. A deficiency of nutrients, especially of nitrogen, limited growth in solutions of 0.08 atmosphere osmotic value supplied at the rate of one liter per plant per day. At the higher rate of supply of the same solution of four liters per plant per day, plants made very good vegetative growth.

3. With nutrient solutions of 0.44, 0.83 and 1.7 atmospheres osmotic value, plants made excellent vegetative growth and had greater total green and dry weight per plant than those in solutions of lowest and highest osmotic value.

4. Vegetative growth of plants with solutions of 3.1 atmospheres osmotic value was good, but was limited by factors other than quantitative inorganic nutrient supply, the most important of which was apparently low available water for tissue development.

5. Fruits set freely on plants grown with nutrient solutions of each concentration.

6. Tomato fruits produced on plants grown with the nutrient solutions of 3.1 atmospheres osmotic value were very much smaller than those produced on the plants grown with the solutions of lower concentration. Many fruits grown with solutions of 0.08 atmosphere were of exceptionally large size.

7. The roots of plants grown with solutions of 0.08 atmosphere osmotic value were brittle, succulent, relatively large in diameter, almost wholly white in color, and appeared to be maturing slowly. The roots of plants grown with the solution of 3.1 atmospheres osmotic value were tougher, less succulent, smaller in diameter, slightly more creamy yellow to brown in color, and appeared to be maturing more rapidly than the roots of the plants grown with the solution of lowest concentration.

8. There was a pronounced difference in the internal water relations of plants grown with the nutrient solutions of different concentrations as evidenced by differences in guttation from the leaves. Under conditions of low transpiration guttation took place abundantly from the leaves of plants grown with solutions of the lowest concentration used, but not at all from the leaves of plants grown with the solutions of the two highest concentrations.

9. The percentage dry weights of fruit, stems, and leaves varied with the concentration of the solutions, the values being the highest in the solution of 3.1 atmospheres osmotic value and lowest in solutions of 0.08 atmosphere.

10. Approximately 80 per cent. of the fruits on plants grown with solutions of the two highest concentrations employed developed the physiological tissue disorder known as blossom-end rot. This disorder consists of the turning brown, shrinking, and death of the tissues at the stylar or blossom end of the fruit accompanying the loss of water from these tissues.

11. No blossom-end rot occurred on fruits grown with the solution of the lowest concentration employed.

12. The development of blossom-end rot was associated with wide fluctuations in the rates of transpiration as indicated by fluctuations in the rates of evaporation and occurred during a period of high transpiration intensities.

13. A slight amount of cracking of fruits occurred under conditions of low transpiration intensities but only in the case of plants grown with the solution of the lowest concentration.

14. There was a difference of approximately four atmospheres between the osmotic values of the extracted juices of similar tissues of plants grown with solutions of lowest and highest concentrations, these values ranging from 6.68 to 10.79 atmospheres in the case of the fruits and from 8.19 to 12.99 atmospheres in the case of the stems.

15. An osmotic gradient of 1.62 to 3.63 atmospheres was found to exist between the extracted juices of the tissues of the fruits and those of the stems and leaves, in the plants grown with solutions of the various concentrations employed. The smallest osmotic gradient occurred in the plants grown with the solutions of lowest concentration.

16. The significance of the differences in osmotic and imbibitional pressures of fruit, and stem and leaf tissues of the plants in the various series

in relation to the incidence and development of blossom-end rot of the fruit is discussed.

17. The importance of the factors of light, temperature, humidity, rate of air movement, and the pH value and oxygen tension of the solution to the development of blossom-end rot of the fruit in solutions of different nutrient salt concentrations are either noted or discussed.

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THE FROST-HARDENING MECHANISM OF PLANT CELLS[1]

G. W. Scarth and J. Levitt

(WITH THREE FIGURES)

Introduction

In the foregoing papers of this series (**25, 26**) we have given an account of our more intensive researches on the physiology of cold resistance, approached always through a study of living cells. We now describe a number of lesser excursions into the same field, and combine with this a survey of the whole problem of hardening in the light of those changes which have been found to accompany it. The relation of proved hardening changes to the mechanism of resistance must remain hypothetical unless we know the type of injury which has to be resisted. This, however, is still a problem, and evidently a complex one. The immediate cause of death is not always the same. Sometimes it is only indirectly related to temperature, as in soil heaving, smothering by ice, and physiological drought. Often there is a time factor which would seem to involve a mechanism different from that responsible for immediate killing.

Though we confine our attention to the more direct and immediate action of frost, the problem is still complicated because, as we shall see, the mode of injury varies with conditions, such as the rate of freezing or the rate of thawing, and also with the type of plant. Very tender plants are killed merely by chilling to temperatures which are still above the freezing point of their juices, or even above 0° C., but most plants of temperate regions suffer no harm unless ice forms in their tissues, and they may be supercooled with impunity. The well-known resistance of dry seeds and spores to the extreme cold produced by liquid air or liquid hydrogen shows that low temperature *per se* is not fatal. There is also some evidence, though rather indirect, that the amount of injury to a particular tissue is more or less proportional to the amount of ice formed in it (**1, 31**). Whatever the mechanism of frost injury, apparently any change which reduces or prevents ice-formation will have a hardening effect, and certain theories of hardening are based entirely on this type of resistance. But tissues do freeze, and the major problem before us is how hardening enables a plant to endure an amount of freezing that is fatal in the unhardened state. It is this problem that depends on the mechanism of injury for its solution, and it will therefore be discussed in relation to theories of the same. These fall naturally into two main groups: Those that regard injury as an effect

[1] Investigation carried out with financial aid of the National Research Council of Canada. This is the third of a series of papers on frost-hardening processes.

of dehydration of the cells; and those that regard it as mechanical. It is convenient, therefore, to classify hardening changes, according to the type of resistance which they seem to offer, into the following categories:

I. Resistance to formation of ice.
II. Resistance to dehydration effects of ice formation.
III. Resistance to mechanical effects of freezing and of thawing.

I. Resistance to formation of ice in tissues

The factors tending to prevent or reduce freezing which are found to become more active with hardening are supercooling, depression of the freezing point, and reduction in the amount of free or freezable water.

Supercooling

Supercooling is always observed when tissues are exposed to freezing temperatures, and has been found (at least in insects) to be greater in hardened than in unhardened tissues (**40**). Generally, however, the maximum extent of the supercooling is but a few degrees, and its duration brief. In such cases it can act only as a first line of defense. But cases of greater and more prolonged supercooling—or pseudo-supercooling—are also on record. Wiegand (**47**) found that in some trees the buds are not frozen at −18° C., and in a smaller number even at −26° C., more than 20° below the freezing point of their cell sap. Lewis and Tuttle (**27**) found that living leaves of *Pyrola* froze at a temperature 28.5° C. lower than that required to freeze dead leaves. Iljin (**21**) says that on account of supercooling, leaves of evergreens, such as *Hedera helix*, often resist temperatures reaching below −20° C. in Central Europe.

In a plant, the conditions for supercooling are well fulfilled as regards breaking up of the liquid mass into separate droplets and capillary columns; but it would seem to be essential for the continuation of the state that the cells be isolated by ice-proof barriers which will prevent spread of crystallization beyond any locus in which it may chance to originate. For this to be maintained there must be very little freezable water in the walls.

In this connection it is significant that *reduction of water content* (which practical men call "maturing") is a regular feature of hardening. The percentage reduction is not usually great, but a small reduction of cell volume can greatly reduce the turgor pressure of the cells and correspondingly greatly increase their suction tension, which of course reduces the water in their walls. Thus, the water which remains in the walls is far below saturation point, and its menisci are retracted into the ultra-microscopic pores, a condition which tends to prevent ice formation. Also, it is known that wilting increases frost resistance, and the presence of free intercellular moisture decreases it. But hardy plants, and animals also, do not

depend upon supercooling for their protection. At a sufficiently low temperature, ice always forms, except in dry objects like seeds, and tissues may be frozen till brittle without fatal effect. In such cases, toleration rather than prevention of freezing is the mode of resistance.

Depression of freezing point: osmotic value

The importance of depression of the freezing point in relation to frost resistance lies not so much in the lowering of the temperature at which ice begins to form—because the difference is only a few degrees at most—as in the reduction in the amount of ice formed, or the increase in the amount of water unfrozen at any temperature below the freezing point. A twice-molar concentration of sugar has a freezing point only 3.25° C. lower than a quarter-molar, but at −15° C. the amount of ice formed is 75 per cent. and 97 per cent. respectively, and the unfrozen water is 25 per cent. and 3 per cent. of the total water. Since at equilibrium "suction tension," and therefore the freezing point, must be the same in every part of a cell—vacuole, protoplasm, and cell wall—the osmotic value of the cells measured plasmolytically tells us the freezing point of the whole tissue. Experience supports theory in this regard. Wiegand's observations prove that ice, though its locus is intercellular, only begins to develop at the freezing point of the cell sap and completely melts at the same temperature. In the following discussion, therefore, we shall refer to the osmotic value or osmotic pressure of the cells rather than to the freezing point of the tissue.

In our attempt to find a correlation between hardiness and osmotic pressure (Levitt and Scarth, **25**), ten species and more varieties were tested. The two tender herbaceous species (sunflower and castor bean) were incapable either of becoming frost resistant or of increasing in osmotic pressure when exposed to "hardening" temperatures. The two semi-hardy herbaceous species (cabbage and clover) showed an increase of 20 to 30 per cent. in osmotic pressure as a result of hardening. The six hardy woody plants possessed maximum winter osmotic pressures up to 400 per cent. greater than their minimum in spring. Among these, the highest concentration was found in the hardiest species, *Caragana,* and it also reached its maximum earlier in the season than did the others. Of the four apple varieties, one of the two hardy ones showed the highest winter osmotic pressure, whereas the other was no different from the two more tender varieties. However, both reached their maxima earlier in the season than the tender varieties.

In short, our results reveal wide seasonal changes in the osmotic pressure, and support the general principle of a correlation between osmotic pressure and hardiness; but they also show that the correlation is not close nor invariable. In particular, they indicate that osmotic pressure rises to a high

value in many woody plants before there is actual need of hardening, and may remain constant during the subsequent period of falling temperature. Unfortunately, we have as yet no tests of the actual hardiness of the plants during this period. An example of the yearly cycle, that of *Hydrangea paniculata,* is shown graphically in figure 1.

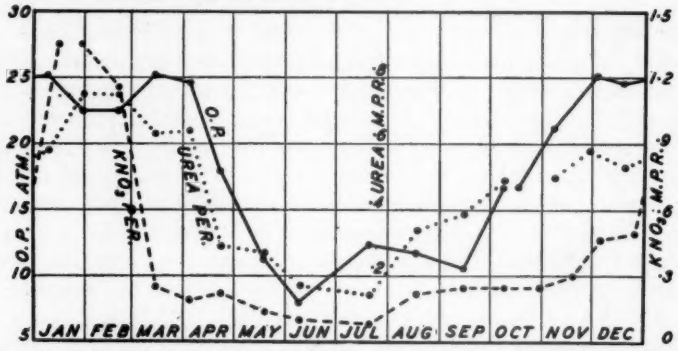

FIG. 1. Seasonal changes in osmotic pressure and urea permeability (Oct. 1934–Oct. 1935) and in KNO_3 permeability (Jan. 1935–Jan. 1936) of cortical cells of *Hydrangea paniculata.*

The depression of the death point, or temperature at which plants are quickly killed, is relatively far greater than the depression of the freezing point of their saps in "natural" hardening, while it is only equal to it in the hardening produced by artificially increasing the sap concentration. AKERMAN found that when the osmotic value of the cabbage cells was increased from the equivalent of 0.4 M to 1.0 M sugar, by allowing the leaves to take up erythrol, the death point was lowered from $-2°$ to $-5°$ C. The change in each is 150 per cent., but we find in natural hardening of the same cells that 150 per cent. lowering of the death point is attended by only 20 per cent. increase of osmotic pressure.

REDUCTION IN FREE WATER

The smaller the proportion of freezable water in the cell, the smaller the amount of potential ice. Furthermore, if free water be synonymous with solvent water, its reduction will raise the osmotic pressure and reduce freezing in that way. The percentage of free water may be lessened in three ways: (*a*) loss of water; (*b*) increase of solids; and (*c*) binding of water.

Loss of water in hardening is usually slight and has already been discussed. Increase of solids must of necessity depend upon nutrition and storage, and cannot result from temperature effect alone; but it may be of great importance in frost resistance. The binding of water requires some discussion.

One of the commonest statements in recent literature on winter hardiness is that an increase in cold resistance is associated with an increase in hydrophilic colloid, or, in other words, a greater proportion of "bound water." The evidence for this statement in the work of ROSA, GORTNER, NEWTON and others, though based on different methods of estimation, is all open to criticism and is opposed by some recent findings (MEYER, **32**; MARTIN, **30**; LEBEDINCEV, **23**). Some authorities, such as A. V. HILL, even assert that there is no bound (in the sense of non-solvent) water even in animal tissues, where we should expect a higher ratio than in plants. When GROLLMAN'S formula (**16**), which makes due allowance for hydration of sugar, is used to calculate bound water in wheat juice from GORTNER'S cryoscopic measurements, the percentage of bound water is found to have a quite small and sometimes negative value. The negative value is explaind by GORTNER (**14**) as due to preferential adsorption of solute.

There are several objections to estimation of bound water on plant juices, among them being difficulties of sampling and possible change, with death, in the hydrophilic properties of the colloids.

With living cells it is not possible to measure bound water directly, but, more to the point, free or solvent water can be determined accurately, and from this, if desired, some estimation of bound water may also be reached. As details of methods and results have been given in a preceding paper (LEVITT and SCARTH, **25**) a summary will suffice here.

If the cell is simply a pure solution surrounded by an osmotic membrane, then it will obey BOYLE'S law, so that $P \propto \frac{1}{v}$. If, however, there is an appreciable quantity of non-soluble solids or of non-solvent (*i.e.*, "bound") water, then this relation will not hold. The formula $P \propto \frac{1}{v-x}$ must then be used, x representing that fraction of the cell volume occupied by non-soluble solids and/or non-solvent water.

In determining which of these interpretations of x is valid, an indirect method must be used. Thus, if x is constant for all values of P, then it represents non-soluble solids, since no change in pressure can alter their volume. If, on the other hand, x varies inversely with P, then it is at least partially composed of non-solvent water, since bound and free water are in equilibrium.

Thus, an estimation of x was found to be useful in determining the relationship between bound water and hardiness. In both hardened and unhardened cells of cabbage $x = 0$ and there is, therefore, no appreciable amount either of non-solvent water or of non-soluble solids in the sap. In cortical cells of *Catalpa* and *Liriodendron*, however, x is large—about 40 per cent. in hardened and 25 per cent. in dehardened twigs. Since it varies with osmotic pressure, x must be partially composed of bound water. Figure 2 allows a

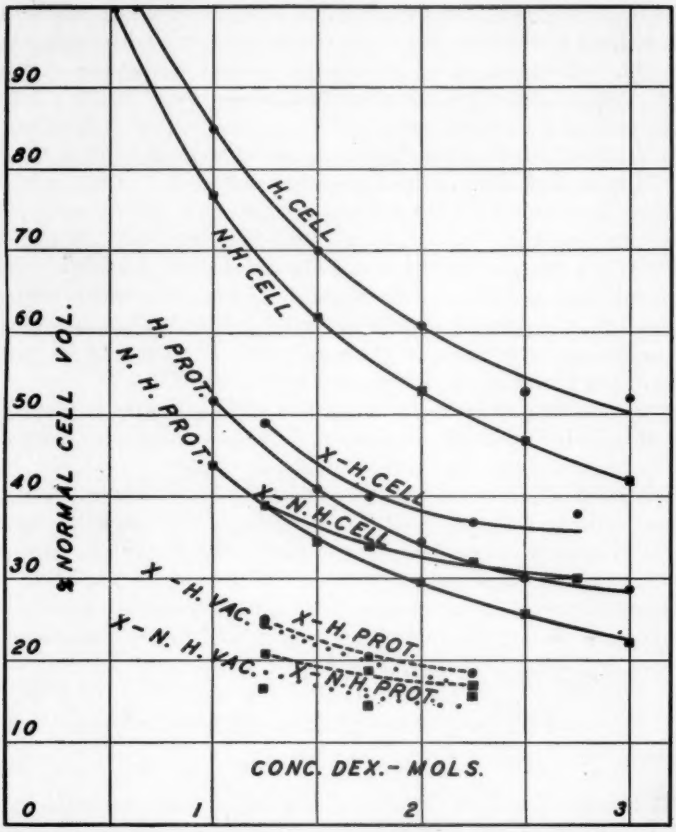

FIG. 2. Change in volume and in non-osmotically active portion (x) of *Catalpa* cortical cells (as well as of protoplasm and vacuole separately) with change in concentration of plasmolyte. The volume of the vacuole is shown by the difference between that of the whole cell and of the protoplasm.

comparison to be made between hardened and dehardened cells as regards the cell volume and the proportion of the cell occupied by x in various concentrations of plasmolyte. The osmotic pressures at normal volume differed only slightly, being 14 atm. in the partially dehardened, and 18 in the hardened; the curves of cell volume tend to diverge in higher concentrations, due to the effect of x. Greater divergence is shown when dehardening is more complete (25). X is proportionally larger in the vacuole than in the protoplasm, and it is here that most of the difference in x between hardened and dehardened cells resides.

Seasonal changes are also significant. Early in May, x drops to half its winter value in both *Catalpa* and *Liriodendron* cells, and since this happens before any appreciable growth, it cannot be due to metabolic utilization of non-soluble solids. Nor can it be caused by a simple hydrolysis of the latter to soluble substances, for in this case a rise in osmotic pressure would result, whereas the reverse occurs. Thus the only possible cause of the seasonal drop in x at this period is a decrease in bound water. A further diminution of x occurs later in the season, but this may be due to the development of new growth.

Briefly then, determinations of the non-osmotically active fraction of the cells of woody plants reveals the existence of bound water which occurs in greater quantity in hardened than in unhardened cells, and which partly accounts for the very high winter osmotic pressures possessed by the former. A semi-hardy herbaceous plant, however, was found to have no measurable amount of bound water either in the hardened or unhardened condition.

The importance of increase of non-solvent space in reducing freezing may be illustrated by a calculation given in a previous paper, which shows that, whereas in non-hardened *Catalpa* 75 per cent. of the cell volume is converted into ice at $-6°$ C., in hardened tissue this amount is probably never reached at any temperature. In view also of the fact that $-6°$ C. is about the critical temperature for unhardened cells (95 per cent. were killed in six hours), the extremely low temperature that the hardened cells endure is not surprising.

Important though this factor may be in extreme resistance, it is not likely to be the only one. Moderate hardening is produced in cabbage without it, and more than can be explained by the osmotic increase. We look, therefore, to other theories of resistance.

II. Resistance to physico-chemical effects of dehydration

Reduction in the amount of solvent water as a consequence of ice formation results in concentration of the cell sap, which many authors, from GORKE (**13**) and LIDFORSS (**29**) onward, have regarded as producing death of the cell by virtue of a toxic effect. More precisely, the mechanism is often pictured as a direct flocculation or even salting out of the protoplasmic colloids by coagulating substances, principally electrolytes present in the sap. There are many theories as to the means by which hardening tends to obviate this result. Protective changes have been detected or assumed, both in the composition of the sap or aqueous phase and in the protoplasmic colloids themselves.

It would be highly desirable to know what happens in the aqueous phase of the protoplasm, as distinct both from the vacuolar sap and from its own colloids, but this is difficult. In dealing with press juices, we may separate

the coagulated colloids from a liquid which, in varying but usually very small part, is derived from the protoplasm but is mainly vacuolar sap. In studying living cells, we may measure properties of vacuole and protoplasm separately but cannot distinguish the phases of the latter.

Since in our own research the approach to the problem is the cytological one, we shall classify the changes according as they appear in the vacuolar sap or in the protoplasm, respectively.

Changes in the cell sap

If freezing kills cells through toxic concentration of the sap, resistance to frost injury might be increased, either by reduction in the amount of toxic substances present or by increase in protective substances.

The agents to which the toxic action has been ascribed are electrolytes in general—in virtue of their precipitating action on colloids—and acid in particular. Protective action has been ascribed principally to sugars as inhibitors of protein coagulation. We shall consider these possibilities in turn.

(a) Concentration of electrolytes.—It has been tacitly assumed by most investigators that any increase in the concentration of the cell sap which occurs on hardening is due to an increase solely of organic solutes. Determinations of electrolytes, when these have been made, have generally failed to show any change (Dixon and Atkins, **9**; Lewis and Tuttle, **27**; Newton, **37**). Recently, however, Dexter (**5, 7**) demonstrated a definite decrease in electrolytes amounting to 50 per cent. (per gm. dry matter) during the hardening period of wheat seedlings, but no change in alfalfa. Greathouse and Stuart (**15**) also have reported a decrease in red clover. We have yet to learn if this is a widespread phenomenon among plants. Dexter himself is of the opinion that the removal of salts by diffusion or any other process does not afford any protection to the plant or its sap. Our own results show that both hardened and unhardened cells can be made to take up very large amounts of KNO_3 without suffering injury. Also the evidence of ash content, even for wheat seedlings (Newton **37**) is against any change in concentration of inorganic salts. The decrease must be in organic electrolytes.

(b) Hydrogen ion concentration.—Schnader and Schaffnit (**45**) put forward the hypothesis, also advocated by Harvey (**17**), that on concentration of the cell sap the H-ions first reach toxic limits and that frost injury is really acid injury. In support of this, Zacharowa (**48**) states that the more acid tissues in a plant die first. Most of the facts, however, are opposed to the theory of acid injury.

Thus, hardening is not accompanied by any significant change in the pH of tissue juices, as was proved by Rosa (**42**), Bakke *et al.* (**2**), Newton

(36), DEXTER *et al.* (8), and DOYLE and CLINCH (10). Our own results show a very slight tendency to reduction of acidity in the sap—a pH change of at most 0.2—in hardened cabbage seedlings. This was found also by DEXTER (6), KESSLER (22), and GREATHOUSE and STUART (15). In no case except some of KESSLER's results is the change sufficient to offer appreciable protection. Since, however, it is possible that pH differences might exist in the respective cytoplasms, unaccompanied by corresponding differences in the sap, we approached the problem from another angle and tried the effect of changing the pH of the cells.

Following SCARTH'S (44) method of altering the pH of living cells, unhardened cabbage seedlings enclosed in bell jars were exposed to the vapor of varying concentrations of ammonia and acetic acid. Solutions of indicators as well as living leaves of *Zebrina pendula* included in the bell jar showed that the series of pH's obtained probably ranged between about 4.4 and 7.5. While still exposed to the vapor, the plants were subjected to frost in a cold chamber. The temperature used (about $-5°$ C.) was sufficient to inflict severe injury on unhardened seedlings, but insufficient to cause much damage to hardened ones. In all cases, both the treated and the control plants suffered between 90 and 100 per cent. injury. KESSLER, allowing plants to take up urea to increase the cellular pH, also found no change in resistance.

Mention may also be made of experiments with expressed juice. First, as found by DEXTER (6) and independently by ourselves (LEVITT, 24), the juice of hardened plants of cabbage is no better buffered and consequently offers no greater opposition to increase of acidity than that of unhardened plants. Secondly, the proteins in the juice of hardened tissue, instead of being more stable toward acid, are more completely precipitated on the acid side of the iso-electric zone than in the unhardened juice. This last point anticipates the discussion of protoplasmic changes, but is mentioned now to complete our argument against the theory of acid injury.

(c) SUGAR CONCENTRATION.—LIDFORSS (28, 29) put forward the theory that the sugars, increase of which is responsible for practically all of the osmotic change in hardening, afford a more important protection than mere osmotic action; namely, a specific protection against coagulation of the protoplasmic colloids, analogous to, but perhaps greater than, the protection which they afford to proteins *in vitro* (NEWTON and BROWN, 38). Whether protoplasm after hardening shows any greater stability, under the action of coagulating agents, will be discussed in the next section. Meanwhile, we may judge the protection theory on the basis of the correlation between sugar concentration and hardiness.

Dependence of some plants upon carbohydrate concentration for hardening seems to follow from the fact that, in the seedling stage when there is no

carbohydrate reserve, they are unable to harden properly if kept in the dark (TUMANOV, **46**; DEXTER, **4**).

On the other hand, as LIDFORSS himself admits, many plants (such as beet and sugar cane) with high sugar content are killed by light frosts, while others (such as mosses and bacteria) with little sugar are highly resistant.

Also, a single species or individual plant may increase the sugar content of its tissue without any increase of hardiness, as shown in the following results.

An experiment of our own with a tender type of plant may be mentioned first. Sunflower plants were grown in the greenhouse for 90 days. The osmotic pressure was low at first (10.6 atm.), later increased (12.4 atm.), and finally, as the flower bud formed, reached its peak (14.3 atm.). Yet neither this increase in osmotic pressure, nor exposure to low temperature, enabled them to assume any frost resistance. The plant is tender at all stages.

Artificial increase of sugar concentration does not produce hardening, even in plants which are capable of it. DEXTER (**6**) showed that when cut leaves of cabbage were set in sugar solution, the osmotic pressure of their cells increased considerably but their frost resistance was not appreciably altered. KESSLER (**22**) obtained similar results, using evergreen plants and glycerine solutions.

From the discussion of osmotic pressure in a previous section, it appears that a correlation between sugar concentration and hardiness is common, but we see from the above that plants may be hardy without high sugar content, and also that increase of sugars apart from other change affords no protection beyond its osmotic effect.

CHANGES IN THE PROTOPLASM

On the theory that death results from dehydration of the protoplasm, the defense mechanism might be either an increase of resistance to the process of dehydration or a decrease of sensitivity to its effects. We shall call these protoplasmic properties hydrophily, and stability, respectively.

(a) HYDROPHILY.—Protoplasm being surrounded by a semi-permeable membrane, its attraction for water is osmotic, but osmotic pressure in turn may be profoundly affected by binding of water on its colloids. This latter phenomenon has been investigated mainly on extracted juice, involving coagulation of the protoplasm, which may obscure any change in the hydrophily of the colloids produced by hardening. Even negative results, therefore, would not disprove the hypothesis that hardening is associated with greater hydrophily of the protoplasm. The question can be settled only by a study of living cells, and we have attempted this in two ways: (1) by comparing the average volume occupied by the protoplasm in hard-

ened and unhardened tissue; and (2) by estimating the bound water from variation of non-solvent space in relation to degree of plasmolysis.

(1) *Comparison of the average volume occupied by the protoplasm in hardened and unhardened tissue.*—Since the total pressure of the protoplasm, in whatever ratio it is divided between swelling pressure and osmotic pressure, must equal the total pressure in the vacuole, an increase in the hydrophily of the protoplasm must result in transfer of water from the vacuole into it until equilibrium is again established. An increase in the volume of the cytoplasmic layer would be the visible result. Of course, changes in the osmotic pressure of the sap must be taken into account, but, allowing for this, measurement of the thickness of the cytoplasm before and after hardening should help to settle the problem of whether its hydrophily changes or not.

We first attempted to make the measurements of the protoplasmic caps in epidermal cells of bulb scales of *Allium cepa* which does not form starch. The result, if there were no complications, pointed to a *decrease of hydrophily in the protoplasm on hardening* (tables I and II).

TABLE I

TOTAL CROSS-SECTIONAL AREA (SQ. μ) OF BOTH PROTOPLASM CAPS (AVERAGE OF 10 CELLS) FROM PLANIMETER MEASUREMENTS OF CAMERA LUCIDA DRAWINGS

ONION NO.	DAYS AT 25° C. (PREVIOUS TO HARDENING)	UNHARDENED	HARDENED	CHANGE
1	7	300	306	+ 6
2	"	303	281	− 22
3	14	376	338	− 38
4	"	477	370	−107
5	28	376	314	− 62
				Average, − 45

TABLE II

COMPARISON OF 10-CELL SAMPLES

ONION NO.	SAMPLE a	SAMPLE b	DIVERGENCE
1	309	319	10
2	370	389	19
3	284	296	12
			Average, 14

The volume of such caps, however, is affected by other factors than volume of the whole cytoplasm—factors such as surface tension of the vacuole, and vacuolar contraction. The result is therefore indecisive.

More satisfactory determinations of volume were made on the cortical cells of apple twigs. These cells possess very bulky protoplasm, occupying about half the cross-sectional area of each cell. They contain no starch in the hardened state, but an abundance of it when in the naturally unhardened condition. Artificial dehardening of branches taken into the laboratory, however, is accomplished before any starch appears, and it was during a two-week dehardening period of this kind that the measurements were made. The average of four branches shows a practically constant volume of protoplasm during this period of dehardening (table III). Since osmotic pressure fell during the same time from 25.5 to 19 atm.—about 25 per cent. reduction—the pressure in the protoplasm must have fallen *pari passu*, indicating, if due to colloidal change, a slight decrease of hydrophily, or conversely a *slight increase of hydrophily* (*though not of hydration*) *with hardening*. On the other hand, the change may be purely osmotic.

TABLE III

TWIGS TAKEN INDOORS FEBRUARY 11, 1935. EACH VALUE IS THE AVERAGE OF 10 CELLS. AREAS OBTAINED FROM PLANIMETER MEASUREMENTS OF CAMERA-LUCIDA DRAWINGS

TIME IN LABORATORY	PERCENTAGE OF CELL AREA OCCUPIED BY PROTOPLASM				
	ALEXANDER	WEALTHY	WOLF RIVER	PATTEN GREENING	AVERAGE
days	%	%	%	%	%
0	47.7	45.2	53.1	47.7	48.4
1	46.3	42.2	53.0	57.4	49.7
3	50.5	46.3	49.5	51.7	49.5
7	52.6	51.1	54.4	49.6	51.6
14	46.7	41.3	50.8	57.6	49.1

Still another comparison was made, using the cortical cells of *Catalpa*. In this case the proportion of protoplasm was found to decrease definitely on dehardening, but the beginnings of growth introduced a complicating factor (fig. 2).

While these results seem to preclude any very great or general increase in protoplasmic volume with hardening, such as the theory of protoplasmic resistance to dehydration would demand, they do not reveal how much of the increase of osmotic value which does occur is due to colloidal change. The next method gives more definite results on this problem.

(2) *Non-solvent space and bound water in the protoplasm.*—It has already been shown that the non-solvent space in hardened cortex cells is

larger than in unhardened cells, and that most of the increase occurs in the vacuole. The protoplasm also possesses a large proportion of non-solvent space—about 50 per cent. of its volume when in equilibrium with a molar dextrose solution, both in hardened and dehardened cells. This is about the same proportion as in the vacuole of dehardened cells, but only two-thirds of that in the vacuole of hardened cells.

Since in the results with *Catalpa*, as shown in figure 2, the volume, both of the protoplasm as a whole and of its non-solvent fraction, is greater in the hardened cells, it follows that the protoplasmic colloids are more hydrophilic.

But even the absence of an increase in the volume of the protoplasm with hardening would not preclude a possible increase of hydration or hydrophilic quality, because of the complication that the dry weight of the protoplasm may diminish. In absence of photosynthesis, there must be a reserve of insoluble material in the cell, which, during the hardening process, is transformed into osmotically active substance and, since simultaneously with the osmotic increase the non-solvent space in the vacuole increases greatly while in the protoplasm it shows an increase only when calculated in relation to the normal cell volume, the diminution of solids is more likely to be in the latter. Commonly, starch is the visible substrate, and that of course is stored in the plastids; but in the starch-free cells, on which our experiments were mostly made, some intermediate reserve carbohydrate or fat may be present in a state of dispersion in the cytoplasm also.

If indeed the protoplasm loses solids to the vacuole, and since its own non-solvent space is undiminished or even increased, the bound water element in it must be augmented. In other words, its colloids become more hydrophilic.

On the same condition, since the volume of the whole protoplasm remains the same, its total water content must be higher.

These volume studies are beset with difficulties and cannot be regarded as more than preliminary, but at any rate on the whole they support the theory of an increase in hydrophily of the protoplasm with hardening.

(b) STABILITY.—This property is closely related to the previous one, since the resistance of the protoplasm, or any other colloidal system, to precipitation or salting out, depends largely on the hydrophily of its least stable elements. But an increase in the hydrophilic property of a portion of the solid phase of the protoplasm would not be reflected in a proportional increase in that of the protoplasm as a whole; so that a great increase in protoplasmic stability may attend only a slight increase in its attraction for water, as estimated by the methods described in the previous section. Also, there are probably factors other than hydrophily concerned in such a complex phenomenon as coagulation of protoplasm.

As a possible clue to the relative stability of protoplasm in the hardened and unhardened state, respectively, we may compare its resistance to other coagulation agents than frost. The action of acid and of heat was tried. Hardened and unhardened cabbage plants were exposed together to vapor of a 7.5 per cent. and also of a 10 per cent. solution of acetic acid for 5 hours. All of the plants suffered, but parts of them remained alive. Hardened and unhardened suffered alike.

The heat test was made by dipping leaves into water at 60° C. long enough to produce partial killing. Again the result was the same in hardened and unhardened plants. *There is no indication here, therefore, of protoplasm becoming less sensitive to coagulating agents in general as a result of hardening.*

HARVEY, who is the author of the theory of greater protein stability, bases his hypothesis on tests with expressed juice. He found (**17**) that when the juice of hardened and unhardened cabbage leaves was frozen and centrifuged, chemical analysis revealed that the precipitation of proteins was greater in the latter. MUDRA (**34**) obtained the same result with other plants. HARVEY also found a greater percentage of soluble amino acids in hardened juice, though NEWTON *et al.* (**39**) regard this as an effect of freezing rather than of hardening.

Testing cabbage juice in relation to the H-ion effect, we obtain the contrary result as regards stability. To a series of tubes each containing 10 cc. of tissue extract, various concentrations of 0.1 N HCl were added. The region of complete precipitation was from pH 3.9 to pH 4.4 in the case of both hardened and unhardened plants. On either side of this zone is a region of incomplete precipitation, the limits of which are pH 5.2 and pH 3.9 in the unhardened, whereas in the hardened plants they extend to pH 5.4 and pH 3.5. Beyond this zone there is little or no precipitation on either acid or alkaline side. *The wider pH zone of precipitation in the juice of hardened plants would point to a poorer stability of their colloids.* This experiment goes to offset results from which a more hydrophilic quality of the juice colloids has been inferred. But of course juice is not protoplasm!

Changes in the colloidal stability of a hydrophilic sol are often paralleled by changes in viscosity, since viscosity is directly and profoundly influenced by the hydration of the internal phase. But the so-called viscosity of protoplasm, as it is measured, is rarely true viscosity, and is influenced by aggregation as well as hydration of particles. Viscosity tests and their significance are reserved for a later section of this paper.

Certain facts are at variance with any theory of injury through dehydration; for example, the experience that drought-resistant plants are not always frost-resistant, and the finding of ILJIN that cells of plants which are sensitive both to light frost and to wilting may survive extreme and pro-

longed desiccation, when this is produced by plasmolysis alone or plasmolysis followed by drying. These phenomena point to mechanical factors as the immediate cause of death, both in freezing and wilting, though not exactly the same in the two cases. Moreover, the most conspicuous of the changes so far described—those producing reduction in ice formation—are as applicable to a theory of mechanical as to one of dehydration injury.

III. Resistance to mechanical effects of freezing and thawing

Theories of mechanical injury may be subdivided into two sets: one which ascribes the effect to pressure of ice crystals, and the other to stress set up by displacement of water. In the former case the fatal period will be that of freezing; in the latter it may be either freezing or thawing. An advantage in the study of mechanical as compared with physico-chemical action is that it can be followed microscopically.

Injury through pressure of ice

The view that frost injury is mechanical dates far back. Duhamel and Buffon (**11**) postulated that ice forming inside a cell ruptured the wall by expansion. From direct observation, however, it was proved by Göppert (**12**) that the walls are not ruptured, and by Sachs (**43**), Molisch (**33**) and others that normally ice forms in the intercellular spaces. Later it was shown that with rapid freezing intracellular development of ice does occur (Müller-Thurgau, **35**; Molisch, **33**). This condition is generally fatal to plant cells (Åkerman, **1**; Chambers and Hale, **3**), whereas some animal tissues are said to be less injured by quick than by slow freezing, because the smaller crystals produce less destruction of the tissue. In plant cells, if the vacuole freezes, the compression of the protoplasm between it and the wall, especially if there is a hull of ice outside (Iljin, **19**), is probably sufficient to cause death, even if the protoplasm itself resists freezing. But opinions differ as to the exertion of mechanical pressure by ordinary intercellular ice. Maximov is a leading upholder of the theory that pressure of the ice crystals on the cells induces coagulation of the protoplasm, and he couples with it the suggestion that mere contact of the ice phase with the plasma membrane may be fatal. On the other hand, Schander and Schaffnit (**45**), followed by Iljin, argue that the ice crystals grow into the intercellular spaces and neither press upon the cells nor even touch the protoplasm. Iljin points out that copious intercellular space in a plant is no protection against frost. Yet it has long been known that cells and tissues may be torn apart by the growth of relatively large ice masses (Pfeffer, **41**), and Wiegand's observations of frozen buds and twigs indicate that less obvious pressure is exerted by crystals of smaller and more ordinary dimensions. There is lack of proof, however, that this is a general cause of injury.

The phenomenon with MAXIMOV first quoted in support of the theory, *viz.*, the protective action of external solutions (which reduced the amount of ice), was shown by ÅKERMAN (1) and ILJIN (19) to depend mainly on plasmolyzing action, and thus pointed the way to an alternative type of mechanical theory.

INJURY THROUGH STRESS SET UP BY DISPLACEMENT OF WATER

WIEGAND (47) and others observed that when tissues freeze in the ordinary way the cells shrink, wall and all, as water is withdrawn. HOLLE (18) noted the same thing in wilting. This behavior is made possible by liquid cohesion within the cell, and adhesion of protoplasm to wall, there being no external liquid to cause plasmolysis. In proportion to the rigidity of the wall, stresses are set up in the cell by its loss of water, and ILJIN observed that in drying cells the protoplasm might be ruptured within itself or torn from the wall—with fatal effect. This he at first regarded as the regular cause of injury, whether in frost or drought, because, when the protoplast was released from the wall by true plasmolysis, the cells could endure severe desiccation and freezing. Later experiments (20) led to a modification of his view, with regard to frost injury at least. He found that application of solutions *after* freezing was also an effective protection, and therefore he assigned injury to the period of thawing. What happens in thawing, according to his observations, is that the walls first absorb water and lift away from the protoplast (pseudoplasmolysis), after which deplasmolysis tends to ensue. He avers that, on account of its high viscosity in the dehydrated state, the protoplast ruptures if deplasmolysis is rapid. Accordingly he argues that the protective function of the solutions bathing the cell is to slow down deplasmolysis. Whether ILJIN'S theory is correct in detail or not, it draws attention to a type of stress to which cells are exposed, and leads us to inquire what kind of changes would enable the cells to avoid mechanical injury under these circumstances.

HARDENING CHANGES IN RELATION TO MECHANICAL INJURY

As already mentioned, some of the changes discussed under dehydration injury are just as applicable here. Part of the osmotic increase is almost always due to lowering of moisture content which, while it reduces the amount of ice and of mechanical injury, is itself a stage in dehydration and not a protection against this result. (*Cf.*, however, its possible rôle in supercooling, as described previously.) The binding of water, while actually a disadvantage as regards undue concentration of the sap, may be very useful against mechanical injury.

The supposed protective action of sugars has also been invoked by MAXIMOV in the case of protoplasmic coagulation from mechanical causes.

But there are certain other properties of the protoplasm which must play a part in resistance to mechanical injury such as we have described, and which have been neglected almost entirely in studies of the mechanism of frost resistance. Such are (a) the permeability and (b) the viscosity of the protoplasm.

(a) PERMEABILITY.—In a previous report (LEVITT and SCARTH, **26**) we have demonstrated that the permeability of cells for polar compounds increases remarkably with hardening and that it undergoes a greater change and is better correlated with hardiness than any other character, even osmotic pressure. Towards apolar non-electrolytes, such as urethane and succinimide, there is no measurable change. Towards polar non-electrolytes, such as urea, thiourea, and glycol, which have relatively small molecules, there is a general relation between permeability and hardening, but the seasonal changes do not always run parallel. Towards an electrolyte (KNO_3) the variation is still greater and the correlation with hardiness much closer. Data regarding water permeability are insufficient to allow of an equally general statement, but as far as they go they show the same trend as with other polar compounds. Cabbage cells are about twice as permeable to water when hardened as when not, and those of woody plants show a greater difference. On the membrane theory of permeability we draw the general inference that there is a widening of the aqueous pores of one or both the protoplasmic membranes of the cell.

Our results show that the correlation between permeability and hardiness exists in various types of plants and shows no exceptions. Also the relation is independent of the nature of the cause which induced the hardening. Low temperature, drought, checking of growth, all increase permeability as well as hardiness.

To some extent the relation seems to hold even in the vegetative, unhardened phase of life. Thus, highly cold resistant cells, such as those of bacteria, mosses, and cortex of woody plants, are at all times and seasons unusually permeable to KNO_3 and perhaps to water.

In view of this widespread association of the two conditions and of the fact that a permeability change does not necessarily attend an osmotic change and is therefore not simply incidental to the latter, it seems likely that there is a direct causal connection between permeability and hardiness. This will be discussed later.

(b) VISCOSITY OF PROTOPLASM.—If, as ILJIN finds, injury may occur through tearing of the protoplast in a phase of thawing out, we might expect to find protection through an increase of plasticity or reduction of viscosity in hardened cells. A comparison of the viscosity of protoplasm in the hardened and unhardened state has been made by KESSLER (**22**), who arrived at the conclusion that viscosity increased, not decreased, in hardening. He

tested the cells of various plants in summer and winter by the centrifuge as well as by the plasmolytic method. The centrifuge method in this case is handicapped by the fact that starch is present in the unhardened and absent in the hardened condition of plants. From subsidiary experiments with darkened plants, KESSLER reached the astonishing conclusion that the specific gravity of starch-free plastids is higher than of those with starch—in spite of the fact that starch is much denser than protoplasm in general, while chloroplasts are believed to be rather lipoidal in composition, and were actually found by himself to have a tendency to move *centripetally* in some hardened tissue. If KESSLER's premise as to specific gravity is at fault, his result that chloroplasts in unhardened plants are more easily thrown down than in hardened is no proof of lower viscosity.

The "plasmolysis time" test was applicable only to *Sempervivum* among the plants tested by KESSLER, where it gave a comparable result. Slower rounding up in the hardened cells pointed to a greater viscosity—or rather a stronger adhesion to the cell wall—in the hardened state. The other genera used, *Hedera* and *Saxifraga,* did not allow comparison, because the protoplast rounded up immediately on plasmolysis.

In our own work on cold resistance, the attempt was first made to distinguish a viscosity difference by observing Brownian movement. However, neither streaming nor any appreciable Brownian movement could be detected in hardened *Catalpa* cells. Nevertheless, the plastids and protoplasm seemed to be clumped together at the periphery and mostly the ends of the cells, perhaps indicating a low viscosity which would allow free play to surface tension. In the dehardened cells, on the other hand, streaming was active and Brownian movement was apparent in the currents. Yet, if the dehardened cells were observed immediately after sectioning, no streaming and little or no Brownian movement was discernible. The protoplasm showed much less peripheral clumping than in the hardened cells. Hardened and unhardened cabbage cells both exhibited Brownian movement, but no difference in the activity could be distinguished.

Plasmolysis shape was next observed. Sections of hardened and dehardened *Catalpa* were placed on a slide in an isotonic solution of $CaCl_2$ (0.30 M and 0.18 M, respectively) which was allowed to evaporate in the air. At the end of four hours the cells were strongly plasmolyzed in both, but the shape differed. The hardened cells were well-rounded, the dehardened ones were strongly concave, adhering to the wall in many places (fig. 3).

In the case of cabbage, sections from hardened and unhardened seedlings were placed in twice isotonic dextrose and examined from time to time. Though the difference was not so striking as in the case of *Catalpa* cells, the rate of rounding up appeared somewhat more rapid in the hardened cells (table IV).

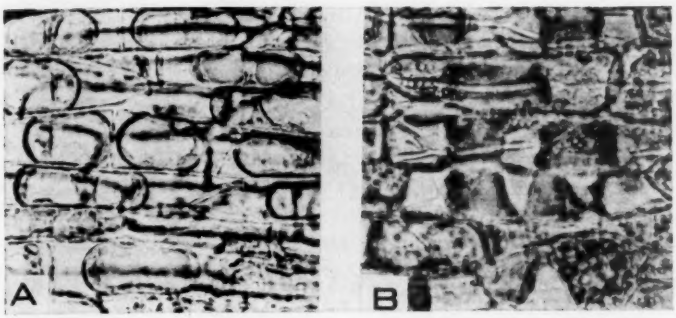

FIG. 3. Plasmolysis shape in hardened (A) and dehardened (B) *Catalpa* cells in isotonic $CaCl_2$ solutions after evaporation in air for six hours.

TABLE IV

RATE OF ROUNDING UP ("PLASMOLYSIS TIME") OF UNHARDENED AND HARDENED CABBAGE CELLS IN TWICE ISOTONIC DEXTROSE. EACH AN AVERAGE OF THREE PLANTS

SAMPLES	NON-HARDENED		HARDENED	
	OSMOTIC PRESSURE	PLASMOLYSIS TIME	OSMOTIC PRESSURE	PLASMOLYSIS TIME
1.	10.0	90	13.6	60
2.	10.6	75	13.6	60
Av.	10.3	82	13.6	60

RESISTANCE TO DEPLASMOLYSIS.—Another mode of experimentation also gave definite and positive results. Sections of hardened and unhardened cabbage plants were compared as regards the ability of the protoplasts to withstand the stretching caused by rapid deplasmolysis. The tendency to injury from this cause generally increases with viscosity of the protoplasm.

First the cells were plasmolyzed for 15 to 20 minutes in twice isotonic $CaCl_2$ and then transferred to distilled water. To determine the number of cells surviving deplasmolysis, the sections were once more transferred to the plasmolyzing solution. The results are presented in table V.

Tender plants always showed sensitivity to deplasmolysis. Thus, *Cordyline* petiole cells almost all burst when transferred to distilled water from twice isotonic $CaCl_2$. In the case of others (tomato, bean), it was found impossible to determine urea permeability, since even this slow deplasmolysis proved fatal.

Hardened and dehardened *Catalpa* cells were then tested, but in their case more severe treatment was necessary to cause injury. Treatments and results are given in table VI.

TABLE V

COMPARISON OF DEPLASMOLYSIS INJURY IN HARDENED AND UNHARDENED CABBAGE CELLS. PLASMOLYZED IN TWICE ISOTONIC $CaCl_2$; DEPLASMOLYZED IN DISTILLED WATER

CONDITION OF PLANT	OSMOTIC PRESSURE (M $CaCl_2$)	PERCENTAGE OF SURVIVING CELLS	
		IN EPIDERMIS AND CHLORENCHYMA	IN PITH
Non-hardened	0.16	trace	0
5-day hardened	0.23	most	0
Non-hardened	0.17	few	0
10-day hardened	0.25	all	many

TABLE VI

COMPARISON OF DEPLASMOLYSIS INJURY IN HARDENED AND DEHARDENED *CATALPA* CELLS

CONDITION OF PLANT	TIME IN PLASMOLYTE (MIN.)	NUMBER OF SURVIVING CELLS
	(a) PLASMOLYZED IN 9 PARTS 2M NaCl: 1 PART 2M $CaCl_2$. DEPLASMOLYZED IN DISTILLED WATER	
Dehardened	60	few
Hardened	360	all
	(b) PLASMOLYZED IN 3M NaCl. DEPLASMOLYZED IN DISTILLED WATER	
Dehardened	15	none
Hardened	15	almost all

Further evidence was obtained indicating that this difference in ability to withstand deplasmolysis injury is even more marked at low temperatures. Thus a much less severe treatment than the above, namely a transfer from twice isotonic to half isotonic dextrose, caused no injury to either at room temperature. Even at 0° C., this procedure had no harmful effect on hardened cells but was fatal to the unhardened, due to the increased viscosity in the latter, which was apparently such as to more than counteract the decreased protoplasmal stress resulting from the reduced deplasmolysis rate.

It is thus evident that hardened cells are more resistant to the ill effects of deplasmolysis than are unhardened cells, and this in spite of the more rapid rate in the former.

Discussion

Reviewing the changes said to be associated with hardening, we have seen that some are not well established and that no single one is adequate to explain the whole phenomenon. Our own research on living cells confirms the importance of certain changes in the sap and brings to light others in the protoplasm. The significance and relative importance of these is difficult

to evaluate from lack of knowledge of the exact mode or modes of injury. The nature of the protection is inferred mainly from the nature of the hardening change.

A wide range of protection is possible with those changes that reduce freezing. Of these, supercooling is not fundamental because hardy tissues survive severe freezing; theoretically, supercooling may even be harmful by leading to intracellular ice development during the quick freezing which follows its breakdown. A rise in osmotic pressure is generally well correlated with hardiness, but in all true hardening it fails to explain more than a fraction of the increased resistance.

Enhancing the osmotic effect in woody plants is a marked increased in "non-solvent space" in the sap vacuole. Part of this is due to insoluble solid—apparently hydrophilic colloid—and part to bound water. This change causes the osmotic pressure to mount more rapidly as the cell contracts and sets a higher limit to the minimum volume which can be reached through water loss. It is calculated that hardened *Catalpa* cells lose no more water through freezing at the lowest possible temperature than the unhardened cells do at only $-6°$ C.

The gelatinous condition of the sap in tree cells may serve another purpose. In ordinary herbaceous plants, intracellular freezing—when it occurs—is located in the sap vacuole. In other words, this is the vulnerable part of the cell as regards the invasion of ice, and such invasion is usually fatal. But in hardy tree cells the vacuole is as rich in hydrophilic colloid as the protoplasm and probably equally protected from freezing even when the eutectic point of the sap is reached.

Important though these factors may be in resistance to extreme cold, they are not the full explanation of hardiness. For example, in the non-hardy state, tree cells still have much colloid in their sap, but they are then more sensitive to frost than hardened cabbage cells which have no colloid and about the same osmotic pressure.

Other changes in the sap have not been shown to be of any great significance. Of those which are supposed to offset the danger of salting out through concentration of the sap, a reduction, with hardening, of the total electrolytes has so far been proved in one or two cases only and disproved in others. Also, in spite of a permeability increase on hardening, there is no evidence of exosmosis from the cells in winter time. Moreover, a high concentration of salt (KNO_3) has been produced inside cells without injury. As regards the theory of toxic acidity of the sap, a reduction of H-ion concentration in hardening has been found in several cases, but only to a slight degree, while artificial alteration of the acidity of the sap is without effect on frost resistance.

The view that the sugars, which are responsible for most of the osmotic change, also exert a specific protection against coagulation of the protoplasm

is countered by the fact that increase of sugar may take place naturally or be produced artificially without a true hardening effect.

As regards changes in the protoplasm, a widely held view is that hardening is accompanied by an *increase of hydrophilic colloid* and consequently of resistance to freezing, to dehydration, and to coagulation.

In attempting to investigate this problem with living cells, in which alone the hydrophilic property of protoplasm is normal, we have seen that in cases where the protoplasm does not swell appreciably in hardening, it may nevertheless be more hydrated if, as seems probable, its insoluble solids diminish. Where swelling does occur the conclusion is more definite.

Evidence regarding changes in the *stability* of the protoplasmic colloids is not decisive. Contradictory results have been obtained with press juices, and with living cells we find that hardening confers no increase of resistance to the action of acid or heat, agents which coagulate proteins.

Altogether, the possibility that frost injury is a physico-chemical effect of dehydration is opposed by the fact that tender cells may often be deprived of water by other means than frost without ill effect. The alternative is mechanical injury. Whatever the precise nature of this injury, the sap changes which tend to reduce the amount of freezing ought to afford some protection; but as we have seen these are not enough.

It would seem that in addition the protoplasm must become more resistant to the mechanical action. Apart from the evidence of a possible colloidal change, which we have just discussed, proof of two very pronounced protoplasmic changes has been educed in the course of our work, *viz.*, an increase in permeability and what may for convenience be termed a fall of viscosity. Let us see if these two hardening changes can be fitted into a scheme of protection against such types of mechanical injury as are known to occur. At least three modes of mechanical injury have been recorded by various authors from direct observation of cells.

Intracellular freezing.—Ice formation within the cell has sometimes been noted and though confined to the vacuole the result is nearly always fatal. The mechanism of injury in this case may be, as Iljin suggests, compression of the protoplasm between the freezing and expanding sap on the inside and the cell wall or a rigid hull of ice on the outside, or it may also be laceration of the vacuolar membrane and other structures by the ice crystals.

At any rate, the condition for internal freezing is that the temperature of the cell sap fall below its freezing point. Ice first starts to form on the cell walls outside the cells, where it normally grows at the expense of water which diffuses from cells. If this keeps pace with the fall of temperature, the resulting increase in its concentration will prevent the sap from freezing, but with a sudden drop of temperature or sudden crystallization as a result of supercooling, the rate at which water can pass out of the cell may be the

limiting factor in deciding whether or not ice will penetrate. Here, then, is a condition when high water permeability may mean safety to the cell.

INTERCELLULAR ICE MASSES.—It is frequently found that when plants which are not hardy are exposed to temperatures slightly below freezing point, large ice masses develop locally in the tissues. These in their growth crush the cells in the neighborhood and may even tear the tissues apart. Since the size of crystals is a function of rate of crystallization, macrocrystalline ice is naturally found only when very gradual freezing takes place, but the more moderate aggregates which develop under other conditions may also caused injury to the cells in contact with them. Conceivably, the rate of crystallization may sometimes depend upon the rate at which water can pass out of the cells, so that the higher the cell permeability to water the smaller the crystals and the less the danger of injury from pressure. It must be admitted, however, that the rate of exosmosis of water from even non-hardy cells is such that this hypothesis of protection is less plausible than that applied to internal freezing.

THAWING.—ILJIN, backed by his experiments, has revived SACHS'S hypothesis of death during thawing. His results cannot controvert the finding of many authors that death may occur in freezing, but they do seem to prove that with the use of salt or sugar solutions, frozen tissues which are still alive may be saved from dying at the stage of thawing out.

Danger would seem to attend both phases of thawing, namely, pseudoplasmolysis, and deplasmolysis. During the former, as ILJIN showed, the water released by thawing is taken up by the cell wall faster than by the protoplast. The former extends quickly to its normal size, while the latter remains contracted for a time. As in true plasmolysis, injury is liable to occur here under certain conditions. If the protoplast adheres to the wall in places, its plasma membrane tends to become disorganized through stretching, or if the external membrane allows water to pass more freely than the vacuolar membrane, the cytoplasm swells and becomes vacuolated. These mishaps in thawing would be avoided if the permeability of the protoplast to water approached that of the wall, and if the permeability of the tonoplast also equalled that of the ectoplast. In other words, increased permeability of the protoplasm, and especially of the tonoplast layer, which normally seems to be the less permeable (HÖFLER), would tend to protect the cell from mechanical injury of this kind. Wall and protoplast would extend together without any pseudoplasmolysis.

According to ILJIN'S later observations, however, the main damage is caused by the rapid stretching in deplasmolysis. The rate of expansion would only be exaggerated by greater permeability to water, but apparently a compensating change in the physical properties of the protoplasm is another feature of the hardening process.

The cells become much more resistant to injury by rapid deplasmolysis

after true plasmolysis, and we may assume that the same would be the case after pseudoplasmolysis in thawing.

The difference between hardened and unhardened cells in this respect is greater at the low temperature at which thawing out occurs than at room temperature. It is probably the result of a lower viscosity of the protoplasm in hardened plants and also a smaller effect of temperature upon its viscosity.

PLASMOLYSIS DUE TO FREEZING.—That cells do plasmolyze sometimes on freezing with formation of ice between the wall and the protoplast is a matter of observation (*e.g.*, CHAMBERS and HALL, 3), but unlike the previous conditions cited, the injury here is hypothetical. Injury in ordinary plasmolysis is common when the protoplasm is highly viscid and adhering to the wall. Low temperature tends to make protoplasm more viscous and hence more liable to injury of this type. As we have seen, the behavior of hardened cells on plasmolysis is exactly such as to reduce this danger. Another advantage of smoother plasmolysis is that strands of protoplasm are less liable to be pinched off by the investing ice.

COLLOIDAL CHANGES IN HARDENING.—The different behavior of hardened cells, both in deplasmolysis and plasmolysis, is such as may be explained by a lowering of viscosity, either of the protoplasm as a whole or at least of its superficial layer. In a visco-elastic material like protoplasm, such a change in consistency is due to gel → sol transformation rather than the true viscosity reduction of a Newtonian liquid. Increased hydration of particles which should increase *true* viscosity may tend towards solation and reduction of *apparent* viscosity. If this should be accompanied by increased hydration of the protoplasm *en masse*, the effect is still more likely to follow.

These are the colloidal changes which our volumetric experiments and other observations have led us to regard as probable. They are also the changes which increase of permeability points to—as regards the plasma membranes at least.

The chemical nature of the colloids involved does not enter into our work, but inasmuch as the osmotic changes are largely produced by carbohydrate transformation, it is likely that the hydrophilic colloid which occupies so much of the sap of hardy tree cells belongs to the same category. This would explain the simultaneous fluctuation of osmotic and colloid substance. The parallel variation of protoplasmic viscosity, and especially permeability, suggests that proteins and lipoids undergo the same type of change as the carbohydrates.

The linked series of changes associated with hardiness as we have described them may be summarized as follows:

1. Complicated hydrolytic breakdown of carbohydrates increases the osmotic pressure of the cell and also in the hardier plants the non-solvent space in the vacuole at the expense of starch and perhaps of other reserves held in the cytoplasm.

2. Due to similar changes in the protoplasmic colloids, the whole cytoplasm, probably, and the plasmic membranes, almost certainly, become more hydrated.

3. As a consequence of this change, the viscosity of the protoplasm is lowered.

4. Because of the change in the membranes in particular, cell permeability is increased.

If all of these changes are causally connected one with another, the correlation of each and all of them with frost resistance would be found, though only one might play a part in it. We have theorized as to the possible rôle of each, but a satisfactory demonstration requires more knowledge of the mechanisms both of injury and of resistance than we yet possess.

Summary

1. Cell changes in hardening are reviewed and theories of the mechanism of frost resistance are discussed. The following are features of hardened as compared with unhardened cells, according to our own results with the plants named:
   - Resistance to injury by deplasmolysis is greater.
   - Rate of rounding up in plasmolysis is greater (*Catalpa*, cabbage, etc.).
   - (Two differences which may point to lower "viscosity.")
   - The relative volume of protoplasm and vacuole changes but little if at all (apple cortex, onion).
   - Resistance to acid and to heat does not change (cabbage).
2. Also the following are features which have been reported in a previous paper:
   - The osmotic pressure is increased, especially in the hardier woody plants (many woody and herbaceous species).
   - Non-solvent space (= colloids and bound water), if present in measurable amount, increases markedly (*Catalpa* and *Liriodendron*), but the increase is greater in the vacuole than in the protoplasm (*Catalpa*).
   - Non-solvent space is practically absent in cabbage, hardened as well as unhardened.
   - Permeability to polar compounds increases, and the more the increase the hardier the plant (many woody and herbaceous species).
3. The press juice of hardened cabbage plants shows:
   - Precipitation of colloids over a wider zone in the pH scale.
   - H-ion concentration slightly lower.
   - Buffering capacity unchanged.
4. Artificial change in the H-ion concentration of the sap in life does not affect hardiness (cabbage).

5. The most pronounced and unmistakable changes in the protoplasm are increased permeability and lowered viscosity; in the vacuole, increased osmotic pressure and (in trees) non-solvent space. Ways are suggested in which all of these protect the cells against mechanical injury due to frost.

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EFFECT OF LIGHT ON SOLANINE SYNTHESIS IN THE POTATO TUBER

HERBERT W. CONNER
(WITH SIX FIGURES)

In 1820 DESFOSSES (**15**) discovered solanine, a basic substance in juice expressed from the berries of *Solanum nigrum*. He found (**14**) it almost insoluble in all solvents tried, except alcohol and acids. Six years later BAUP reported (**5**) it also to be present in the potato (*Solanum tuberosum*), but he found much more in the sprouts than in the tubers.

The chemical nature of the substance is even yet only partially known. ZWENGER and KIND (**51**) found that it is stable when boiled with potassium hydroxide, and that it cannot reduce Fehling's solution, but (**50**) that it can be hydrolized by strong acids, giving rise to salts of an alkaloid (solanidine) and a solution which is able to reduce Fehling's solution. Identification of the hydrolytic products has been a gradual achievement of several investigators. It appears (**24**) that a molecule of solanine is composed of one molecule each of solanidine, glucose, galactose, and rhamnose; and that three molecules of water are split out in the formation of the glucoside whose constituent groups are linked in the order in which they have been mentioned (**35, 49**). The empirical formula of solanidine is still a matter of disagreement (**6, 35, 42, 46, 49**), but it is known to be a tertiary base with a secondary alcohol group (**42, 46**).

The glucoside is interesting for several reasons:

1. It is the only basic-reacting glucoside known (**31**).[1]

2. It has the foam-producing and haemolytic properties of saponins, yet contains nitrogen.

3. It frequently occurs in poisonous concentrations in market potatoes.

4. It is claimed that lesser concentrations increase intestinal absorption (**28**) by precipitating sterols (?).

5. It is uniquely toxic to conidiospores of *Cladosporium fulvum* which have been raised on glucose agar (**1, 41**) although spores taken directly from infected tomato leaves tolerate it better.

6. It contains two sugar groups that have not been found free in the plant. If the availability of rhamnose or galactose, or both, limits the elaboration of solanine then a study of the factors which increase the amount of glucoside formed may indirectly contribute to an understanding of how monosaccharides, other than glucose, arise.

[1] Since neither solaneine, nor any glucoside of solanthrene has yet been isolated in crystalline form, the hypothetical solanine group of glucosides is here referred to as "solanine."

To determine in what tissue the solanine is formed and stored, a number in the parenchyma and around the fibro-vascular bundles. By allowing the the most commonly employed procedures. Using such a method, ALBO reports (3: p. 199) that solanine is found relatively in abundance in active meristems of all parts of the plant, and that it disappears from inactive tissues. In the tuber he claims that it is

"particularly localized in the periderm (phellogen), although it is also found in the parenchyma and around the fibro-vascular bundles. By allowing the tubers to sprout, it is found very diffusive in the sprouts, and it is also observed in the pith and in the subepidermal cells of the roots."

All such color tests involve the use of concentrated sulphuric acid (2), usually warmed. Under such conditions solanine is partially hydrolized to solanidine. Since solanidine also gives these color reactions, HANSEN concluded that such tests cannot be reliable. Color changes of anthocyanins when acid is added offer further complications.

To minimize these difficulties BREHMER (9) tried preliminary washing of cut sections with water, ether, and alcohol. Such a method should reduce the troubles from undesired colors, but it also allows leaching out of solanine. HANSEN tried to measure solanine by its haemolytic activity, but was not able to get quantitative results. FISCHER (18) showed that the rate of haemolysis varies greatly with the change in the acidity of the medium. Using buffered blood-gelatin strips a quasi-quantitative micromethod was developed by FISCHER and THIELE (19) which indicates solanine, as distinct from solanidine. They report no solanine in the periderm of the tuber, most of it being in the ten outer layers of cells of the storage tissue, but at any depth they find more near a sprout than farther away. In the sprouts they find both solanidine and solanine in the region outside the cambium, but only about $\frac{1}{8}$ as much solanine and no solanidine in the central portion. They conclude that solanidine is first elaborated in an actively growing tissue and is later condensed with sugars to form solanine.

MORGENSTERN made a notable advance in gravimetric methods (34). His procedure involves extraction of a sample of ground tissue with water and warming the extract with acetic acid. After the filtrate has been evaporated to a syrup, hot alcohol is added. The filtrate is then evaporated to dryness, and is leached with hot acetic acid water. The filtered solution is heated with an excess of ammonium hydroxide. The precipitate is leached with hot alcohol, and the filtrate evaporated to dryness. The residue is dissolved in hot acetic acid water, and the solanine in the filtrate is precipitated with ammonium hydroxide and collected on weighed filter paper.

BÖMER and MATTIS have modified the procedure of MORGENSTERN somewhat. They recommend (8):

1. Extracting the ground sample four times with acetic acid water and combining the extracts.
2. Heating the extract with an excess of ammonium hydroxide and evaporating to dryness with diatomaceous earth.
3. Extracting the residue with three or four aliquots of boiling alcohol, alternated with repulverizing in a mortar.
4. Filtering the combined extract and evaporating to dryness.
5. Dissolving the residue in dilute acid and boiling the solution with an excess of ammonium hydroxide.
6. Filtering off and dissolving in hot alcohol.
7. Evaporating the filtrate to dryness and dissolving in dilute acid.
8. Reprecipitating with hot ammonium hydroxide and collecting on a weighed filter paper.

Analytical considerations

Gravimetric methods are tedious and involve so many manipulations that loss of solanine is almost inevitable, in preparing a white precipitate. In a tissue bearing both glucoside and free alkaloid, each will be extracted and the weight of the final precipitate will represent the sum of solanine plus solanidine.[2] Since considerable amounts of the free alkaloid may occur in potato tissue (JORRISSEN and GROSJEAN report (**26**) up to 0.15 per cent. in fresh sprouts), it would seem that a method of analysis would be desirable which would distinguish between the two substances and which would yield more accurate results than the blood, gelatin method of FISCHER and THIELE.

In solanine analysis, either hot ethyl alcohol or, more commonly, a dilute acid is used for extraction. Precipitation is accomplished by evaporation, or by addition of alkali. In these manipulations solanidine and certain magnesium and calcium impurities behave similarly to solanine, making purification possible only at the expense of loss of the product desired for weighing. Weighing introduces further difficulties, for solanine dries to constant weight very slowly. (A 5.3544-gm. sample of Merck solanine spread in a weighing bottle less than one centimeter deep dried to constant weight in an 80° C. oven at one-half atmospheric pressure only after twelve weeks. Moisture content 10.0 per cent.) A large number of filtrations is objectionable for aqueous solutions which have been made alkaline, since excess of alkali, and other factors not completely known, tend to produce a colloidal form of solanine which passes through the filter paper, or at best to produce a gel which clogs the paper, making each process tedious, and often requiring several papers for one filtration.

[2] To verify this, solanidine was prepared from Merck solanine by hydrolysis with 2 per cent. hydrochloric acid and decomposition of the crystalline hydrochloride with alcoholic potassium hydroxide. The base, recrystallized from ether, was found to be well soluble in alcohol and in 0.2 per cent. acetic acid although slowly so at room temperatures.

Extractions can be much simplified by allowing acid rather thoroughly to leach the ground tissue and then pressing quite dry in a hydraulic press. For this purpose a cylinder from an automobile engine, containing two piston heads (the upper one bearing a connecting rod and the other cut off at the second ring and perforated with several holes on the top) makes a satisfactory press cylinder, the pressed material being held in a linen bag between the pistons. A portion of the cylinder head provides support for the lower piston and drainage for the juice through a sparkplug hole into a beaker beneath. By this means it is possible to get out in one leaching practically all of the solanine extractible by the four separate processes of the BÖMER and MATTIS method.

To simplify the procedure and to avoid estimating solanidine as solanine, an attempt was made to estimate the extracted solanine volumetrically, obviating the necessity for weighing, and, more important, the necessity for eliminating the contaminating compounds whose solubilities are so like that of solanine. This is possible where solanine is estimated from the amount of sugar which is split off by acid hydrolysis, for none of these contaminants affect alkaline cupric ion. Because of the small amounts of sugar available in any one analysis, it was found expedient to use a modification of the PAVY reagent (**36**) with methylene blue as an internal indicator (**30**) to sharpen the end point. The alkalinity was increased in order to maintain at least one-half normal sodium hydroxide after addition of all the sugar solution, as seems advisable from the work of QUISUMBING and THOMAS (**38**), to keep a high ratio of cuprous oxide to sugar. The new procedure is simple and expeditious, save for the first filtration, which may be somewhat slow.

In the separation of tuberin from solanine, it is necessary that no solanine should be hydrolized by heating in dilute acetic acid. In a test of this, samples of one-half gram were boiled for one-half hour in 25 cc. of 0.2 per cent. acetic acid. No appreciable reducing ability developed, a confirmation of the work of ZWENGER and KIND (**51**), and of BÖMER and MATTIS (**8**). Indeed, solanine is unique in the conditions requisite for significant hydrolysis. COLOMBANO (**12**) claimed no appreciable hydrolysis at room temperatures for this concentration of solanine in 2 per cent. hydrochloric acid, even after a month's standing. The writer finds that after standing five months only 23 mg. of "glucose" appears under these conditions from a 500-mg. sample. In contrast, heating with 2 per cent. hydrochloric acid gives a fairly complete and rapid hydrolysis. HEIDUSCHKA and SIEGER (**24**) find over three-fourths of the solanine hydrolized in one-half hour at the boiling point.

Analytical procedure

A sample of not much more than one kilogram of fresh tissue is ground in a Russwin mill, and the juice and solid material just covered by 0.2 per

cent. acetic acid. After standing for about six hours, the liquid is strained through a linen bag, the residue being pressed out in a Carver hydraulic press, using a pressure of more than 35 kilograms per square centimeter. This expressed fluid is heated on a water bath to precipitate the tuberin, filtered through paper pulp, the pulp washed out with a few more cubic centimeters of acid, and the filtrate made alkaline by a few drops of ammonium hydroxide. This on further warming precipitates the solanine. It should be left in the evaporating dish overnight to allow large flocs to form. These are collected on a filter paper, in a Büchner funnel using suction, and washed with a few cubic centimeters of water containing a few drops of ammonium hydroxide. The volume of the filtrate is determined and the weight of solanine which it contains is computed, using the value 25 milligrams per liter (**29**).

For estimation, the precipitate is leached through the paper into a 50-ml. volumetric flask, by 25 ml. of 2 per cent. hydrochloric acid, and kept at a gentle boil on a hot plate for one hour. While hot, 10 ml. of 5 per cent. ammonia water is added, and after cooling, the whole is made up to 50 ml. with water. The reducing power of the filtered solution is determined by copper reduction.

The copper reagent consists of three grams of copper sulphate pentahydrate dissolved in 600 ml. of concentrated ammonium hydroxide, added to 1,000 ml. of Fehling's B, and the whole made up to 2,000 ml. with water. Such a solution shows no auto-reduction on standing. A 10-ml. aliquot is raised to boiling in a 300-ml. Erlenmeyer flask, with a drop of 1 per cent. aqueous solution of methylene blue. The flask is fitted with a two-holed rubber stopper, one hole for steam exit, and the other for the offset tip of a burette which is used to run in the sugar solution (the hydrolysate). Titration is complete on loss of the red color.[3] These titration values were standardized, using oven-dried samples of Merck solanine. Using the proportions indicated, at least 15 mg. of solanine should be present in the precipitate. Otherwise, the sugar solution formed is such a weak reducing agent that a large amount of solution is necessary to complete the titration. As it runs into the flask a significant amount of oxygen may also enter. Preferably a precipitate should contain less than one-half gram of solanine because of the danger that more concentrated hydrolysates may bump during the boiling. As shown by figure 1, intermediate values (20–100 milligrams) can be determined with greater accuracy.

Total solanine is obtained by adding the amount precipitated to the amount left in the alkaline filtrate, unprecipitated.

[3] The presence of ammonium ion in the sugar solution, added *during* the boiling, avoids one of the difficulties in the PAVY method, namely, precipitation of cuprous oxide if boiling is prolonged enough to drive off too much ammonia before the titration is complete.

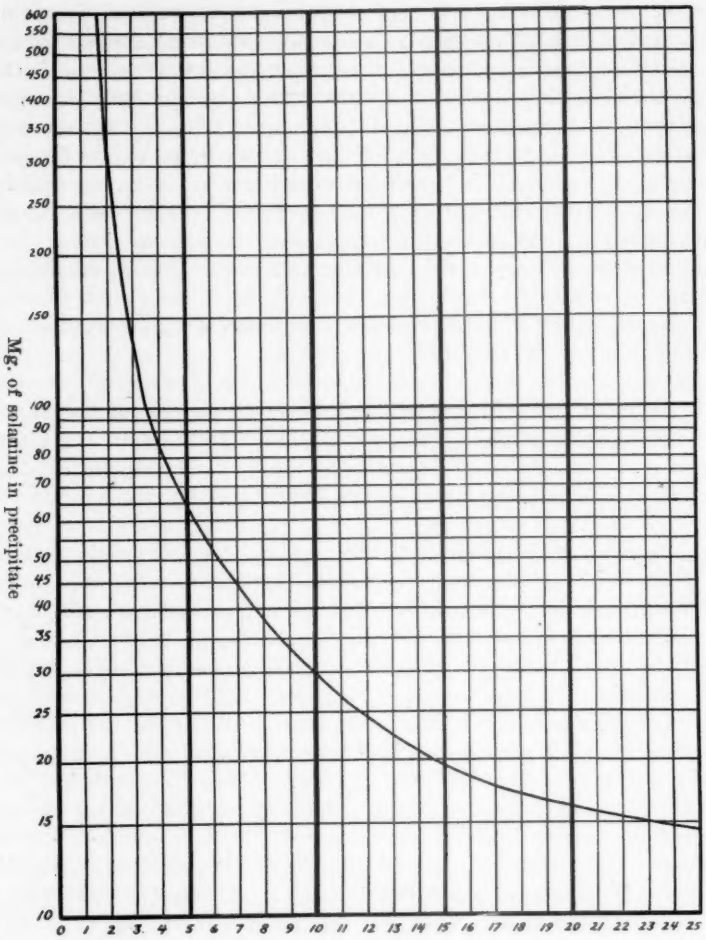

FIG. 1. Copper reduction values of solanine hydrolysates.

Effect of light on tubers

Since solanine-rich tubers are frequently green, and since "sunburned" tubers are uniformly bitter and have an increased solanine content, the matter of tuber irradiation has received the attention of several investigators (**7, 19, 21, 22, 34, 43**). MATTIS found (**7**) that the metabolic activity was quite significant. Old tubers in storage showed very little response to

irradiation, while fresh tubers, when plowed out and exposed to direct sunlight, increased up to more than ten times their previous "solanin" content.

No investigation has been reported which has considered the relative significance of the various wave length bands of sunlight for solanine formation. Since the initial rise in solanine content commonly precedes visible chlorophyll synthesis (**21, 22**) it might be inferred that the former is not a consequence of the latter, but any convincing demonstration must involve the isolation of a selective radiation as a result of which no greening accompanies solanine elaboration. This paper describes an attempt:

(1) to increase the solanine content of tubers without chlorophyll elaboration; and

(2) to determine whether under equivalent intensities the wave lengths which chlorophyll ordinarily utilizes in glucose synthesis are the ones which are most effective in solanine synthesis.

Equipment

Seven bottomless wooden boxes were constructed, 33×33 cm., 18 cm. high; the side walls were covered with white lead enamel, a good reflector for wave lengths from 0.3 μ to well beyond the visible (**32**: p. 88). The top of each box consisted of a set of four identical Corning glass filters, 16.2×16.2 cm., whose margins were sealed with adhesive tape. An area of approximately 850 sq. cm. remained exposed to the radiation of the tungsten lamp overhead. These seven filters showed transmission peaks in various parts of the spectrum. The transmissions of these glasses were determined over the entire wave length range up to 4 μ, using a quartz spectrograph with sector attachment for the ultra-violet, a spectrophotometer for the visible, and a spectrometer with rock salt prism and thermopile for the infra-red.

Three additional boxes were prepared of sheet iron, 38×48 cm., 30 cm. high, with tops which were tight-fitting removable trays 8 cm. deep. The bottom of each tray had a rectangular cutout 32×42 cm. A sheet of ultra-violet transmitting glass was fastened into each tray bottom with pitch. A cooling coil of copper tubing, carrying a stream of tap water, was fitted just inside the tray wall. The coil and the metal portion of the tray were given two dippings in melted paraffin. Care was taken not to let paraffin overlap more than about 3 mm. onto the clear portion of the glass. Curved false bottoms were placed in each of the three boxes and two of the boxes were placed on platforms of unequal height, so that while the three box bottoms were horizontal, they were at sufficiently different heights to keep the three false bottoms as portions of one large cylindrical surface of 30-cm. radius, whose axis was the tube of an overhead Cooper-Hewitt mercury arc in Uviol glass, which had to be kept on a downslope for proper operation.

TABLE I

CHARACTERISTICS OF THE FILTERS EMPLOYED WITH MAZDA LAMPS

FILTER			LAMP		RADIATION	
DESIGNATION	MEAN THICKNESS	APPROXIMATE TRANSMISSION LIMITS	DISTANCE	WATTAGE	CHARACTER	DESIGNATION
	mm.	μ	*cm.*			
			53.6	100	Visible approximates artificial lighting but weak in yellow band which distinguishes IV from V; infra-red intense;	I
91B	7.62	0.365–4.2				
			57.8	1000	ultra-violet slight	VIII
			24.5	100	Visible like sunlight;	II
G90A	5.23	0.344–4.0			infra-red considerable;	
			51.8	1000	ultra-violet slight	IX
			29.6	100	Visible largely green;	III
G124JA	4.44	0.356–4.2			infra-red slight; ultra-	
			41.2	1000	violet slight	X
G24	4.27	0.600–4.1	33.4	100	Visible only red and orange; (should be efficient for glucose synthesis); infra-red considerable; ultra-violet absent	IV
G34	3.96	0.520–3.6	44.1	100	Visible red, orange, and yellow (should be efficient for glucose synthesis); infra-red considerable; ultra-violet absent	V
		0.460–0.620	34.0	1000	Visible green; infra-red	VI
G401CZ	4.85	and			intense; ultra-violet	
		1.1–3.6	23.8	1000	absent	XI
		0.350–0.480			Visible blue; infra-red	
G53C	4.75	and	27.0	1000	slight; ultra-violet	VII
		1.5–3.6			relatively intense	

Equal portions of the lamp were partitioned off for each filter by sheets of tin plate, as shown in figure 2.

Into one of these trays was placed a 5-cm. layer of an aqueous solution containing 290 gm. of $NiSO_4 \cdot 6\text{–}7H_2O$, and 83 gm. $CoSO_4 \cdot 7H_2O$ per liter. The second tray contained a 5-cm. layer of an aqueous solution containing

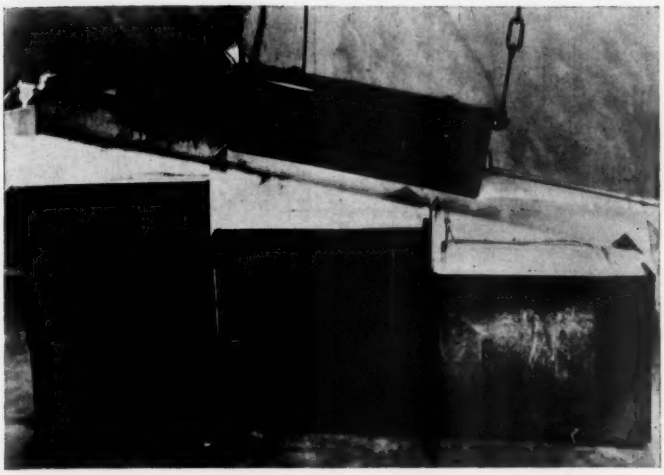

FIG. 2. Mercury arc lamp and metal boxes showing the three tray box tops with cooling coils and the four sheets of tinplate partitioning the lamp.

8.8 gm. $CuSO_4 \cdot 5H_2O$ and 300 cc. of concentrated NH_4OH per liter. The third tray held a 5-cm. layer of an aqueous solution containing 200 gm. of $NaNO_2$ per liter. Radiation entering these three boxes had to pass through the 5-cm. solution layer, the sheet of ultra-violet transmitting glass in the bottom of the tray, and in the case of the Cu filter an additional thickness of ultra-violet transmitting glass which was used as a cover. The transmissions of these filters was computed from measurements made on the solutions and on a sample of the glass, using a quartz spectroscope and a photo-electric cell. Figure 3 indicates the wide dissimilarity in opacity of these three filters. Although available emission data on the 450-watt Cooper-Hewitt arc[4] was limited to that furnished by BUTTOLPH (**10**) for a new lamp, it may be concluded that tubers under the $-NO_2$ filter received almost no ultra-violet (radiation XIV), tubers under the Cu filter received long wave length ultra-violet and short wave length visible light (radiation XIII), and that the tubers under the Ni-Co filter received no visible radiation but did receive a much more intense exposure to wave lengths of about 0.3 μ than in the other two cases.

The tungsten lamps employed with the Corning glass filters were standard, internally frosted, 115-volt Mazda type C lamps operated at a potential of 113 volts (mean of a 100 hr. graphic record). When new, the energy distributions of their emissions were approximately as shown in

[4] Previously operated for about 1000 hours.

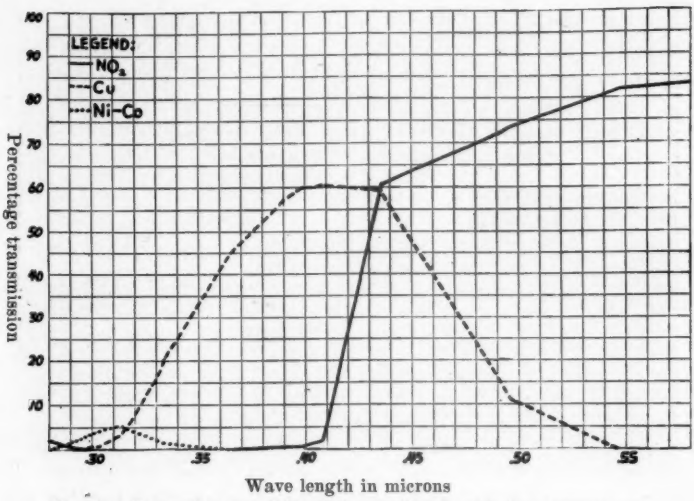

FIG. 3. Transmissions of the filters employed with the mercury arc.

figure 4. With use the emission decreases, but much more rapidly at the short wave length end of the spectrum. To minimize this error in the experiments reported, lamps were used for only 100 hours before replacing with new ones over filters which transmitted below 0.45 μ.

For each wave length the intensity of the lamp source was multiplied by the percentage transmission of the filter in question, and graphed, using a linear scale. Total energy of wave lengths shorter than 0.69 μ was read from this graph, using a planimeter. Knowing that the total radiation of a new 1000-watt lamp was 798 watts, and that of a 100-watt lamp 70.7 watts, and knowing that for the lamps used the intensity of the radiation was about

TABLE II

CHARACTERISTICS OF THE FILTERS EMPLOYED WITH MERCURY ARC LAMP

FILTER		RADIATION	
DESIGNATION	APPROXIMATE TRANSMISSION LIMITS	INTENSITY MAXIMUM	DESIGNATION
	μ	μ	
Ni-Co	0.28 – 0.37	0.30 and 0.31	XII
Cu	0.30 – 0.54	0.43	XIII
$-NO_2$	0.40 – 1.0	0.55	XIV

21 per cent. greater than average, for the cone of light within 48° of the lamp axis (**44**: p. 22), it was possible to compute at what distance a lamp should be placed in order to have any desired intensity on the far side of an interposed glass filter. For computation, the distance from the filament to the center of one of the four glass plates was considered to be the average distance.

For radiations I, II, III, IV, V, VI, and VII these distances were made such that the energy of wave lengths less than 0.69 μ getting through a glass filter would be 100 microwatts per sq. cm. for normal incidence. This

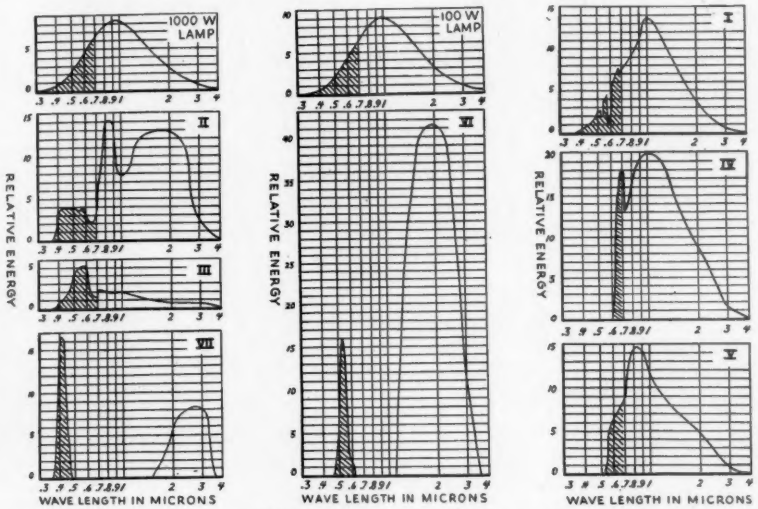

FIG. 4. Spectral energy distributions of radiations I, II, III, IV, V, VI, VII, and of the tungsten lamps employed. The equal energy areas are shaded. Wave lengths are shown on a logarithmic scale in order to make minor differences more apparent in the range which is significant for photosynthesis.

intensity value was chosen quite arbitrarily. The 0.69-μ limit was selected as approximately the upper limit of glucose and chlorophyll synthesis (**13, 37, 39, 40**). Tubers were placed in a single layer on the floor, covered with a filter box, and irradiated from an overhead lamp. Where 1000-watt lamps were employed, the reradiation from the glasses made a 21° ± 2° temperature control at the tuber level possible only after installing compressed air lines to ventilate the boxes.

For radiations VII, VIII, IX, X, and XI, the filter boxes were clustered on edge about a single 1000-watt lamp. In each box the tubers were held in a single layer between two pieces of ½-in. wire mesh, and the box back

was covered with a light-tight pan. Two holes were cut through the bottom side and one in the top for the insertion of elbow pipe fittings so that ventilation could be accomplished through a suction manifold to a vacuum cleaner without entry of extraneous light.

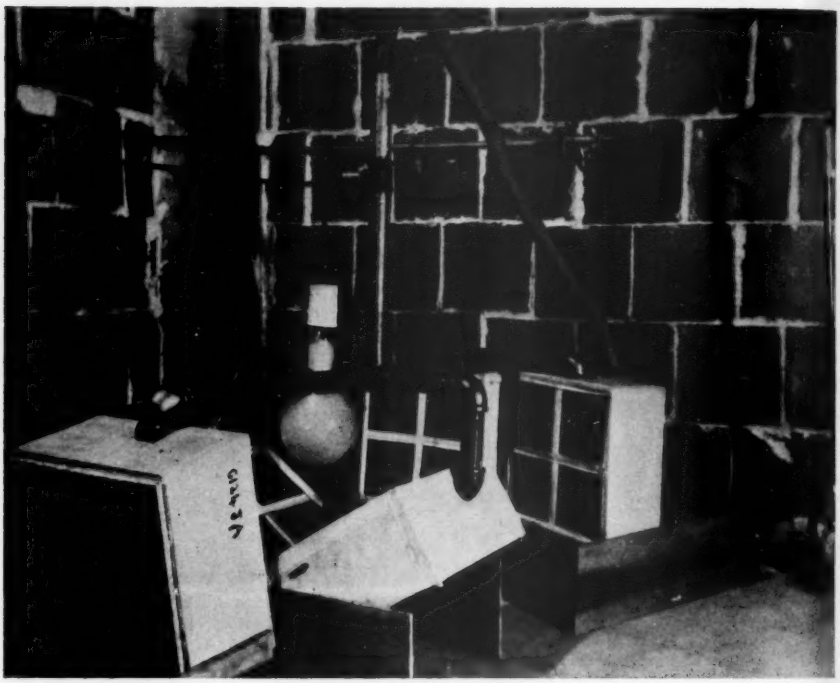

FIG. 5. Arrangement of Corning filters for radiations VII, VIII, IX, X, and XI.

From the results obtained with the previous irradiations, it seemed desirable to compare radiations which were of equal energy in the short wave transmitting band of filter G53C. At the 100-microwatt per sq. cm. intensity this was impractical for filter G401CZ since its transmission in this band (0.35–0.48 μ) was so low that it would have to be placed so close to the lamp that it would subtend too large an angle at the filament (with resultant gross uneven intensities getting through differing portions of the filters). Instead, the G401CZ filter was placed at a distance such that the lower half of its visible transmission band (below 0.53 μ) let through the arbitrary intensity of 100 microwatts per sq. cm.

The angle of incidence of the light rays to the glasses varied from 0° to a maximum of about 46° for the closest placed filter. Within this range the reflection from glass surfaces is uniform (**25**: p. 1572) but the absorption

which occurs in the glass increases with the increasing angle of incidence. Since the transmission measurements were made only for normal incidence, the peaks in figures 4 and 6 should be somewhat more intense than shown.

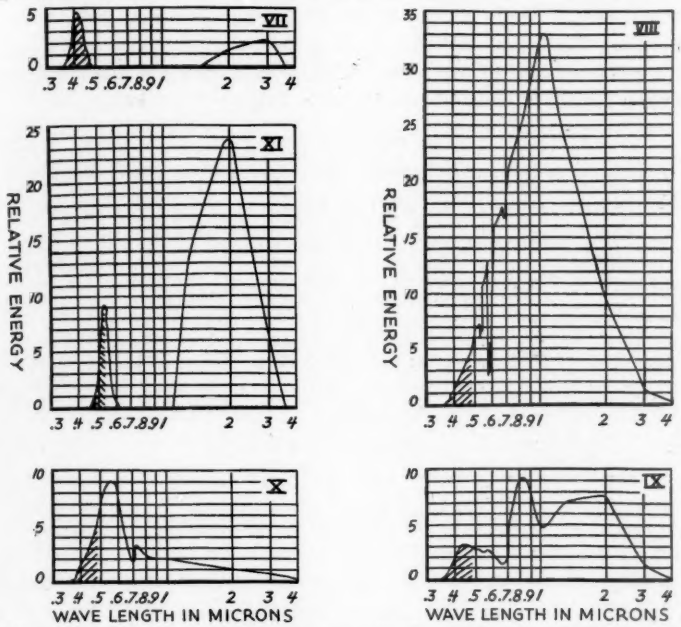

FIG. 6. Spectral energy distributions of radiations VII, VIII, IX, X, XI. The equal energy areas are shaded. Energy units are the equivalent of four of the energy units of figure 4.

Bliss Triumph potatoes were used. The "dormant" tubers were furnished by the State Agricultural Experiment Station at Spooner, Wisconsin. They were dug after sundown, in September, and kept continuously in darkness at room temperature for the two weeks prior to irradiation. This gave some opportunity for storage metabolism to be approximated. The "active" tubers, obtained in May from a dealer at New York Mills, Minnesota, were free from dormacy, having been dug by daylight the previous September and placed in a cool dark storage bin the same day. They were kept in the dark at room temperature for one month, the sprouts off, and the tubers irradiated.

Experimentation

SUNBURNED

"Sunburned" tubers (in which the washing away of soil has exposed them on one side to sunlight for an indefinite number of days) were har-

vested at maturity of the crop and immediately placed in a dark container. After four months' storage in the dark, the short (1–5-cm.) sprouts were removed and the tubers separated into two easily distinguishable portions, green and red, in every case cutting toward the center of the tuber from the boundary of the green zone. Both samples were intensely bitter.

Using the method of analysis described in this paper the solanine contents were found to be as shown in table III.

TABLE III

SOLANINE CONTENT OF SUNBURNED TUBERS AFTER 4 MONTHS OF STORAGE

SAMPLE		SOLANINE			
DESIGNATION	WEIGHT	AMMONIA WATER SOLUBILITY	PRECIPITATE	TOTAL	PERCENTAGE CONCENTRATION
	gm.	*mg.*	*mg.*	*mg.*	%
Red tuber	1001	11	20	31	0.0031
Green tuber	1015	9	24	33	0.0032
Sprouts	995	4	500	504	0.0507

DORMANT

Normal tubers were placed 30 cm. beneath the bare mercury arc lamp for a 20-day continuous exposure. Other tubers were exposed to radiations I, II, III, IV, V, VI, and VII, in each of which the total energy of wave lengths shorter than 0.69 μ was 100 microwatts per sq. cm.

A titration end point was not obtained for the hydrolisates from the precipitates of samples I, II, III, IV, V, VI, and check (indicating less than 10 mg. of solanine in any precipitate).[5] While the exact concentration in these samples remains uncertain, it is certain that the tubers receiving radiation VII contained at least 50 per cent. more solanine than the tubers under any of the other Corning filters. The analytical results are given in table IV.

ACTIVE

Normal tubers were continuously exposed for twenty days to radiations VII, VIII, IX, X, and XI, in each of which the total energy of wave lengths shorter than 0.48 μ was 100 microwatts per sq. cm. Other tubers were given twenty days of exposure to radiations XII, XIII, and XIV. Table V indicates the results obtained.

[5] However, there is no assurance that any precipitate contains solanine unless the titration is complete; the precipitate might be solanidine with calcium and magnesium phosphates only.

TABLE IV

SOLANINE CONTENT OF DORMANT TUBERS AFTER 20 DAYS OF CONTINUOUS RAYING

SAMPLE		SOLANINE			
DESIGNATION	WEIGHT	AMMONIA WATER SOLUBILITY	PRECIPITATE	TOTAL	PERCENTAGE CONCENTRATION
	gm.	*mg.*	*mg.*	*mg.*	%
Bare arc	437	25	22	47	0.0108
Dark (check)	276	5	<10	<15	<0.0026
I	601	7	<10	<17	<0.0028
II	543	4	<10	<14	<0.0026
III	625	3	<10	<13	<0.0021
IV	626	4	<10	<14	<0.0022
V	626	6	<10	<16	<0.0026
VI	799	3	<10	<13	<0.0016
VII	666	5	26	31	0.0047

For the tubers under the last three radiations, color distinctions were marked. Chlorophyll was absent in XII and XIII, and abundant in XIV. Anthocyanin was notable in XII, hardly noticeable in XIII, and not visible in XIV.

TABLE V

SOLANINE CONTENT OF ACTIVE TUBERS AFTER 20 DAYS OF CONTINUOUS RAYING

SAMPLE		SOLANINE				
DESIGNATION	WEIGHT	AMMONIA WATER SOLUBILITY	PRECIPITATE	TOTAL	PERCENTAGE CONCENTRATION	INCREASE OVER CONTROL
	gm.	*mg.*	*mg.*	*mg.*	%	%
Beginning	1725	14	30	44	0.0026	− 7
Dark	1197	6	28	34	0.0028	0
VII	1132	11	48	59	0.0052	+ 85
VIII	1096	10	93	103	0.0094	+236
IX	1128	7	96	103	0.0091	+225
X	1061	6	16	22	0.0021	− 25
XI	1158	5	25	30	0.0026	− 7
XII	1148	10	76	86	0.0076	+171
XIII	1135	13	34	47	0.0041	+ 46
XIV	1197	13	33	46	0.0038	+ 36

Discussion

The concentrations reported here (by the volumetric method) are in general much lower than those obtained by gravimetric estimation of

"Solanin" (including solanidine) (**7, 33, 34**). BÖHME has reported that the normal content of potatoes (not sunburned) may be as much as 0.015 per cent., five times the amount found by the writer in very bitter, sunburned tubers, but BÖHME has reported other normal tubers with concentrations as low as 0.0020 per cent. GORTNER (**20**: p. 466) gives an average value for normal tubers of 0.0024 per cent., which agrees well with the $<$ 0.0026 per cent., 0.0026 per cent., and 0.0028 per cent. values obtained by the volumetric method.

Perhaps more work has been done on anthocyanin formation than on any other type of glucoside synthesis which is induced by radiation. In a recent summary of the field ARTHUR (**4**: p. 1116) concludes:

> "There is a general agreement among various investigators that the blue-violet and often the ultra-violet regions of sunlight are especially effective in anthocyanin production."

Since radiation XII was so much more effective than more intense radiations XIII and XIV, it may be concluded that the ultra-violet band is of more consequence than the blue-violet in the production of potato anthocyanin.

A comparison of qualitatively different light sources by equating total energies gives quite a misleading result where the significant radiations are only a small portion of the total energy, *e.g.*, the visible radiation of a Mazda lamp. This has been a serious error in the studies of KNIEP and MINDER (**48**) and of WURMSER (**13**). By equalizing energies below the 0.69 μ limit, a better comparison was possible as to photosynthetic efficiencies. Greening, and glucose synthesis are most efficient in red-orange light (**13, 47, 48**). Since the most effective increase in solanine occurred upon irradiation by source VII it seems that a glucose synthesis cannot be a necessary intermediate. VII has no red, orange, or short infra-red, but neither has VI. VII lacks green, but so also does IV. The unique formation of relatively large amounts of solanine under radiation VII would not appear to be connected with the omission of any repressant rays, but rather with the presence of rays which induce the synthesis. Of the two possible bands, blue-violet—ultra-violet, and longer infra-red, the latter is ruled out since VII is intermediate in intensity between VI and III as regards these wave lengths.

To test whether the short wave band was responsible, the series of radiations with active tubers was tried. If short rays are effective, then increasing the intensity of I, II, and III to the same level as in this band (VIII, IX, and X) should also show increases in solanine content in tubers that are metabolically active. Whereas radiations I and II did not produce any notable effect on the solanine content of dormant tubers, the same sources were much more effective than VII on active tubers when the inten-

sity was sufficiently greater to give comparable amounts of the short end of the spectrum (VIII, IX). X did not show any increase. Whether green rays have a specific effect in the repression of solanine formation, as induced by short rays, has not been investigated, but the significance of short rays has been confirmed by the results obtained with the mercury arc. XIV contained almost no rays shorter than blue; it induced the least glucoside synthesis. XIII consisted of green, blue, and long ultra-violet; it gave intermediate effect. XII consisted of ultra-violet only, and was much stronger than XIII or XIV below 0.32 μ; it produced the greatest solanine increase. Incidentally it induced no chlorophyll formation.

This study has not been extended to the shorter ultra-violet or to the long infra-red beyond the emission limits of the Mazda lamp since such radiation probably cannot penetrate (**11, 27, 45**) to the tissues in which solanine is formed (**19**). It lumps as "solanine" all substances which precipitate out with the true solanine and which have—or which on acid hydrolysis split off—copper reducing groups. Solaneine (**17**) and the glucoside of solanthrene (**16**) are included, if they occur, but so far as now known they are closely related to solanine in the metabolism of the tuber and they are present only in minor amounts.

Summary

1. A new analytical method has been developed for the quantitative estimation of solanine in the presence of solanidine, based upon the amounts of sugars set free on acid hydrolysis.

2. Upon irradiation by a mercury arc in Uviol or by Mazda lamps, potato tubers increased in solanine. This was accompanied by the appearance of anthocyanin in the sprouts.

3. Wave lengths which are efficient for glucose synthesis did not induce a significant increase in solanine, but did result in an increase of chlorophyll.

4. Ultra-violet rays of about 0.3 μ are effective for solanine formation but not for chlorophyll elaboration.

The writer wishes to express his gratitude to Dr. W. E. FORSYTHE for computing the emission of the Mazda lamps employed, to the Spooner Experiment Station for the tubers studied, to Dr. F. P. ZSCHEILE for measuring the aqueous filters, and to Professor C. A. SHULL for extending valuable suggestions and criticism.

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METHODS OF RESEARCH ON THE PHYSICAL PROPERTIES OF PROTOPLASM[1]

WILLIAM SEIFRIZ

(WITH FIVE FIGURES)

Much excellent research has been done with a test tube and a Bunsen burner, but certain problems cannot be successfully attacked without the aid of intricate apparatus. It is the latter type of research, in so far as it applies to studies on the physical properties of protoplasm, with which this report deals.

Obviously, the first instrument with which the student of protoplasm must become familiar is the microscope. It had its beginning in the primitive use of lenses by the ancients. In the first book devoted exclusively to microscopical studies, "Micrographia," which was written by HOOKE in 1660, his compound microscope is illustrated (fig. 1).

It is not within the scope of this report to present a detailed description of the compound microscope. A knowledge of this instrument is extremely useful and it is suggested that the diagrams and descriptions of their optical systems appearing in the catalogues of leading microscope manufacturers be studied.

The microscope has a limit fixed, not by the magnifying power of lenses, but by the wave length of light and other factors which determine its *resolving power*. The highest powers of the microscope reveal objects which are about 0.1 μ (0.0001 millimeter) in size.[2] Greater magnification could be obtained, but it would be of no use, because it would then be impossible to distinguish between two particles or lines which are closer together than the length of the wave of light used. It is not greater magnification, but

[1] One of the purposes of this committee is to make available those methods of physical measurements which have proven useful and have passed their preliminary experimental stages. However, all methods should be regarded as steps in the progress of research which undoubtedly will be modified and improved from time to time as new facts are discovered. Obviously these reports cannot be exhaustive treatments. They are intended to point out fundamental principles and include a bibliography which should serve as a starting point for those interested in a more comprehensive study. In the present report of the physical methods committee, organized for the committee by Dr. SEIFRIZ, the immediate objective has been to discuss briefly the approved methods and apparatus used in determining the physical properties of protoplasm and cell structure.—EARL S. JOHNSTON, Chairman.

[2] μ = micron = 0.001 millimeter
mμ = millimicron = 0.001 micron
μμ = micromicron = 0.001 millimicron
Å = Ångstrom unit = 0.1 millimicron
μμ is often erroneously used for mμ.

FIG. 1. The microscope of ROBERT HOOKE (1660).

greater resolving power that is needed. Resolving power is the capacity of a lens to separate one line or point from another lying very close to it. Let us take a specific case: A diffraction grating, as used for the formation of spectra, may contain 1000 lines to a millimeter. Microscope lenses of the highest power easily distinguish these as individual lines clearly separated from each other. But if there are two lines where there was but one before, then each line will be separated from its neighbor by 0.5 μ. It would, in this case, be barely possible to distinguish individual lines if the microscope lenses and the eye of the observer were of the best. We can go no further with direct vision. As a result, efforts to increase the powers of the microscope have of late been directed toward methods of illumination.

Illumination

Two lines of attack on the problem of microscopic illumination have been followed; either light of other wave lengths is used, or white light is applied in different ways. The light, other than composite white light, so far most successfully used is ultraviolet.

THE ULTRAVIOLET MICROSCOPE

The resolving power of a lens is proportional to its numerical aperture and inversely proportional to the wave length of the light used. In other

words, the greater the numerical aperture and the shorter the wave length of light, the smaller the structure which can be imaged by the lens. Written in the form of an equation:

$$\text{Smallest structure visible} = \frac{\text{wave length of light}}{\text{numerical aperture}}.$$

The average length of a wave of white light is 0.55 μ (5500 Å); accordingly, a lens with a numerical aperture of 0.65 will reveal an object $\frac{0.55}{0.65}$ μ or 0.85 μ in size. This means that if the lens is used to examine parallel lines 0.5 μ apart, the image produced would not contain separate lines, the structure would not be resolved, and higher magnification would be of no avail.

The resolving power of a lens increases with the angle at which light enters it, and with the refractive index of the medium intervening between the lens and the object viewed. The increase due to the angle of the entering light is proportional to the sine of ½ of this angle. If the maximum angle at which light can enter is 80°, the sine of ½ of 80°, or 40°, is 0.64. If the medium between lens and object is water with a refractive index of 1.33, the resolving power will be 33 per cent. greater than if it is air, the refractive index of which is 1.00; and if the medium is cedar-wood oil with a refractive index of 1.51, the resolving power will be 51 per cent. greater than in the case of air. ABBE, German optician, introduced the term "numerical aperture" (abbreviated N.A.) to include both of these factors. The numerical aperture is the product of the sine of ½ the maximum angle at which light enters the objective, and the index of refraction of the medium between the front lens of the object and the cover glass. To increase the resolving power of a lens, we can (as the above formula shows) decrease the wave length of light, or increase the numerical aperture. The latter has been done by lens manufacturers as far as practicable, with 1.6 as the upper limit. As the numerical aperture is increased, chromatic and spherical corrections are more difficult, and the working distance of the lens becomes less. We then turn to the possibility of using light of shorter wave length. Blue light is shorter than the average (yellow) light of the sun. It should, therefore, increase the resolving power of lenses. Ultraviolet light, having shorter wave lengths than the blue, should serve the purpose still better. For this reason ultraviolet has been used, and the method has given rise to the *ultraviolet microscope*.

By using ultraviolet light with a wave length of 275 mμ, as compared with 550 mμ, the average wave length of white light, it is possible practically to double the resolving power of a lens. The theoretical limit of the resolving power of an objective of numerical aperture 1.40, is 0.16 μ for a wave length of 450 mμ, and 0.13 μ for a wave length of 365 mμ. This theo-

retical value of 0.13 μ is only slightly above the lower limit of microscopic visibility. An objective of numerical aperture 1.40, with the 365 mμ ultraviolet spectral line (of mercury) will give 19 per cent. more resolving power than the best microscopic system with white light. But there are difficulties. Glass is practically opaque to light waves of less than 300 mμ, *i.e.*, to ultraviolet light; furthermore, such light is invisible to the human eye. The first difficulty is overcome by using quartz lenses, and the second by substituting the photographic plate for the human eye.

The ultraviolet microscope was presented to the biological world some thirty years ago by KÖHLER of the Zeiss scientific staff at Jena. The instrument has a potential resolving power twice that of the best optical systems using visible light, which means that it gives twice as much detail as do the best lenses with ordinary light. It also gives greater optical differentiation, *i.e.*, greater contrast, for example, between the translucent, glass-like parts of a cell. It thus obviates the need of staining protoplasm, which is difficult of accomplishment in living matter. The method was originally intended for metallographic studies, but in spite of a promising future, little outstanding work was done with the ultraviolet microscope. LUCAS (**14**), of the Bell Telephone Laboratories in New York, has revived its use.

Ultraviolet light of a known wave length is obtained by passing light from a suitable source, such as a spark between cadmium or magnesium electrodes, through a quartz prism which resolves or breaks it up into its component wave lengths. Ultraviolet rays of about 275 mμ wave lengths are selected. As these are invisible, the optical image cannot be seen in the ordinary way. It must either be photographed or visualized on a fluorescent screen. The fluorescent screen commonly used is made of uranium glass, and is mounted interchangeably with a camera just above the microscope. Magnifications of 5000 diameters result in sharp brilliant images with a degree of resolution surpassing by far that achieved with any other known optical system. With such an equipment, LUCAS has taken some excellent photographs of living cells.

Any wave length shorter than the average of white light will increase the resolving power of a lens. Consequently, light *just beyond* the visible limit of the violet end of the spectrum (the visible spectrum stops at about 390 mμ) should be better than white light, and it retains the advantage of white light in passing through glass. Ultraviolet of 365 mμ is, therefore, used with glass lenses.

The use of ultraviolet light for photographing living tissues is open to criticism because of the harmful effects which it may have on the cells. Certain microorganisms are destroyed almost instantly by ultraviolet light; others are mildly excited, and still others remain normal.

Vertical illumination

Direct light, *i.e.*, rays coming from below the microscope stage, is the time-honored method of illuminating transparent material through the microscope. Obviously, opaque material cannot be so viewed, consequently it must be illuminated from above. The types of apparatus designed for illumination from above are numerous; the following are representative. The illuminator of W. A. Beck (fig. 2) is a simple and inexpensive optical

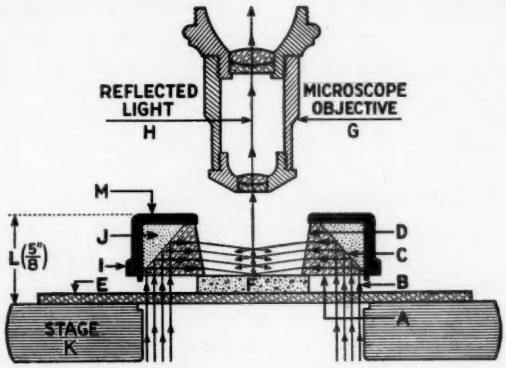

Fig. 2. The Beck illuminator. Light enters from below, is totally reflected by the prism C, and directed down upon the opaque material F.

system in which light comes from below, enters a circular glass prism from the upper silvered surface of which it is totally reflected to the opaque material lying in the center on the microscope stage. Similar, and as simple, is the "epi-mirror" of Carl Zeiss; it is part of the "epi-" equipment of Zeiss for dark-field illumination by incident light. One of the earliest of vertical illuminators is that invented by Silverman (manufactured by Spencer Lens Co.); instead of a reflector or prism, the light source is directly above the material surrounding the objective. Of a like design is the surface illuminator of Bausch and Lomb. It consists of a ring of electric lights surrounding the objective. Other more expensive devices for vertical illumination are the "Ultropak" of Leitz, and the "epi-condenser" of Zeiss. These latter instruments use light from a small and concentrated electric source placed close to the objective and entering it from above.

The vertical illuminator is a development of a metallurgical microscope which is used to advantage mostly in connection with the observation of highly-reflecting surfaces, such as those of polished and etched metals. Introduced by the metallurgist, the method has been adopted by the biologist (**32**) who finds it of advantage in studying not only opaque but also transparent material by viewing it in another light.

Dark-field illumination

When Faraday showed the Royal Society a suspension of colloidal gold, he told them that the solid particles of gold in suspension scattered light rays which strike them, so that a brilliant cone of light is produced. *Dark-field illumination* and the *ultra-microscope* are based upon this phenomenon. Colloidal matter is illuminated laterally against a dark background. Microscopically invisible particles thus become "visible" because of the light which they scatter. The direct beam of light from the source of illumination does not enter the eye of the observer; only the scattered rays from the particles are seen. The colloidal particles thus illuminated appear as brilliant spots against a black background. Two main types of lens systems are used to give lateral illumination—the cumbersome equipment known as the *ultra-microscope,* and the simple *cardioid condenser* or *dark-field illuminator.*

The development of the ultra-microscope is due to the Germans, Siedentopf and Zsigmondy. The instrument designed by them is a large and expensive apparatus which others have attempted to simplify. This type of ultra-microscope is sometimes referred to as the slit microscope, the slit being a small aperture corresponding to the hole in the shutter of a darkened room into which a beam of light enters and illuminates the motes in the air. The ultra-microscope consists further of a series of lenses and apertures, apart from the microscope proper, which direct a light ray into the colloidal material and thus illuminate it laterally. The Tyndall cone of a colloidal solution, as seen with the naked eye, is the total effect of the scattering of light by many particles, no individual particles being visible. In the ultra-microscope the individual particles are "seen" as centers of bursts of light. Thus viewed, a colloidal solution resembles the Milky Way at night, but with every "star" dancing about in active Brownian movement.

The simplest way to obtain a dark field with indirect illumination is shown in figure 3. Another simple method is the central stop diaphragm to be used with an Abbe condenser. It excludes all rays within the field of the objective's aperture. A dark field may also be produced quite easily by removing the top part of an Abbe condenser and substituting for it a dark-field element. The usual optical system for indirect illumination is the dark-field condenser. The same fundamental principles underlie this instrument as those of the slit type of ultra-microscope, namely, a black background, and indirect illumination. The light, however, enters the condenser from below as in an ordinary microscope.

There are several types and modifications, such as the *cardioid,* the *paraboloid,* and the *change-over* condenser. The first two names indicate the nature of the curve of the reflecting surface. The light rays strike and are

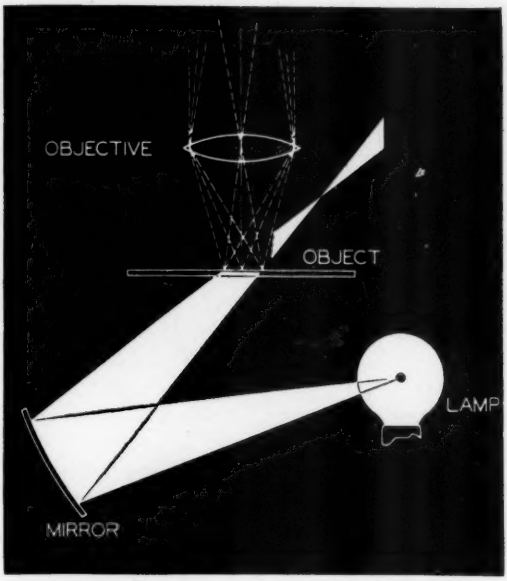

FIG. 3. A simple arrangement for dark-field illumination. (From Bausch and Lomb.)

reflected by two successive mirrored surfaces which direct and concentrate them at a point in the colloidal solution (fig. 4). Thus does the illuminating beam of light not enter the microscope, only the scattered rays from the colloidal particles being visible. The change-over condenser permits

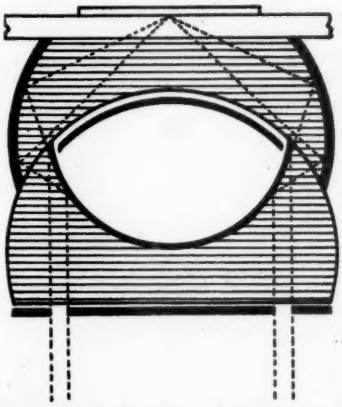

FIG. 4. The optical system of a cardioid dark-field condenser.

going from direct illumination, as in the ordinary microscope, to dark field without changing condensers.

A special dark-field condenser is manufactured by Bausch and Lomb which in itself takes the form of a moist chamber; it has been designed for work in microdissection.

The Spierer lens

The Spierer lens (**26, 28**), an ingenious development of dark-field illumination (o, fig. 5), is based on the principle that a colloidal particle scatters

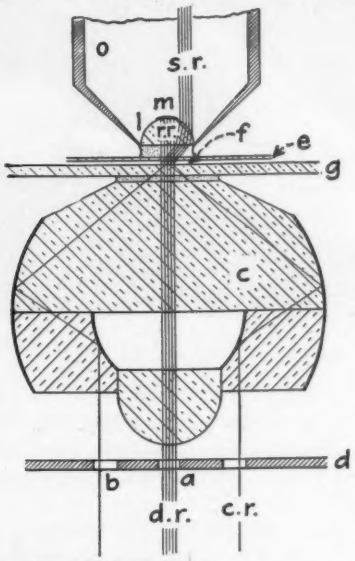

Fig. 5. The optical system of a Spierer lens:

o = objective
m = Spierer mirror
l = lens
r.r. = reflected rays
s.r. = scattered rays
e = cover slip
f = material
g = slide
c = cardioid condenser
d = diaphragm
a = aperture for direct rays
d.r. = direct rays for Spierer lens
b = slot for cardioid condenser rays
c.r. = cardioid condenser rays

light unevenly, *i.e.*, there are more rays given off in one direction than in any other, namely, from that side which is away from the source of illumi-

nation. Degree of visibility of submicroscopic particles thus depends upon the angle of the illuminating ray. If a colloidal particle is viewed *toward* the source of illumination instead of at right angles to it, as in the slit ultra-microscope, or at 45 degrees to it, as in the dark-field (cardioid) condenser, the observer will then see the maximum amount of scattered light. A brighter picture, smaller particles, and a finer structure will consequently be discernible. In order to accomplish this, SPIERER placed a tiny metal (silver, gold, platinum, or aluminum) mirror (m, fig. 5) within an oil-immersion objective. This metallic reflector covers but a small part of the lens surface. Light comes directly from below, and passes through the colloidal matter. It then enters the lens, where it strikes the mirror from which it is reflected downward, thus illuminating the particles a second time from above. The first illumination is, however, the important one for it directs the maximum brilliancy of the ellipse (or rather the three-dimensional ellipsoid) of scattered light into the lens. In the usual dark-field condenser a less bright portion of the ellipse enters the objective.

The presence of a mirror in the lens prevents direct light from entering but does not hinder the entrance of scattered light around it. In order that only so much direct light shall enter as can be reflected by the mirror, the aperture of the iris-diaphragm below the microscope stage must be as small as the mirror. The SPIERER lens (o, fig. 5) is a dark-field system in itself; it may, therefore, be used with an ordinary Abbe condenser if the diaphragm is closed to a pinhole. But it is more convenient to use a special cardioid condenser (c, fig. 5) which has a fixed aperture (a, fig. 5) of correct size (*i.e.*, as small as the mirror, m, fig. 5). The use of a cardioid dark-field condenser adds still another ellipse of scattered light.

All dark-field methods of illumination depend upon diffraction phenomena for their usefulness. This is both an advantage and a disadvantage. The diffraction of light by ultra-microscopic structure makes that structure evident but the picture seen may not be an exact duplicate of the real one. It may be merely a diffraction image. This is not necessarily, nor always, true. One must, therefore, be particularly cautious in interpreting dark-field pictures. One can, however, always be certain that a diffraction image at least indicates that structure is there and usually it suggests the type of structure, if it is not an exact counterpart. With the aid of dark-field illumination, structural features in protoplasm not visible with direct light are made evident. Innumerable tiny particles in active Brownian movement are to be seen in protoplasm, as well as in the vacuoles of cells. TAYLOR (30) has determined the sign of the electrical charge on the ultra-microscopic particles in the protoplasm of a slime-mould; the particles were made visible by a cardioid condenser.

Ordinary light-field illumination usually shows the protoplasmic substratum to be homogeneous. Dark-field (SPIERER) illumination shows this apparently optically empty matrix to be a very fine emulsion consisting of two phases which have been termed *cryptoplasm* (hidden), *i.e.*, the optically empty background or matrix, and *phaneroplasm* (visible), *i.e.*, the emulsion globules. When once shown to be present by dark field, structures such as the fine protoplasmic emulsion can often be found with light field. SCARTH (**22**) has photographed the fine protoplasmic emulsion.

Tandem and inverted microscopes

Biologists often try new combinations of lenses, sometimes to the distraction of the physicist who fails to find any optical reason for the new combination. But the biologist finds it works better that way and so goes happily on; thus two microscopes may be used in tandem, the second picking up the image of the first. This permits the use of a low-power lens on the first microscope and therefore greater working distance between lens and object. Greater magnification is obtained through the second microscope, but the resolving power of the complete system is determined by the resolving power of the first objective.

E. LEITZ has designed, at the suggestion of CHAMBERS, an inverted microscope: the objective occupies a position under the cover glass, while the fluid drop lies upon it. This establishes conditions which greatly favor the technique of micromanipulation.

Vision at high speed

HARVEY and LOOMIS (**12**) devised a set-up which permits viewing a living cell while it is being centrifuged at high speed. The microscope, of course, is stationary. As the material passes under it, a light flashes on just for the moment so that one obtains an apparently continuous picture made up of many rapid successive exposures precisely as in the cinematograph. The gradual precipitation of granules due to centrifugal force can thus be observed while it is going on.

The polariscope

The physical (optical) properties of crystals differ in different directions. An object seen through certain crystals (*e.g.*, Iceland spar) appears to be double. This is known as *double refraction*, and is believed to be due to the *polarization* of light. Ordinary light vibrates in all planes extending out from the point of propagation, but the vibrations of polarized light are restricted to one plane. If a crystal polarizes light, it will allow light already polarized to pass through in one plane only, and two crystals will permit polarized light to pass through only if their planes of polarization

are parallel. If two such crystals are crossed, more or less of the polarized light will get through, depending on the angle of rotation. When the crossed crystals are at right angles to each other, no light can pass through. Such crossed crystals when built for the study of polarized light are known as *Nicol prisms;* mounted in an instrument they constitute a *polariscope.* The presence of double refraction and the polarization of light by crystalline matter may be detected and studied with the polariscope. The two prisms, the *polarizer* and the *analyzer,* are inserted, the one below the microscope objective (usually in conjunction with the condenser) and the other above the ocular, the crystalline substance being placed between them on the microscope stage. Rotating one of the prisms, which is mounted on a scale, gives in degrees the angle of the plane of vibration. If the material is not crystalline there will be no decrease in light intensity as the Nicol prism is rotated.

By means of the polariscope, chlorophyll within a living cell has been found to be crystalline, and as it is fluid, it must be of the nature of a liquid crystal. FREY (**10**) has ascertained the crystalline structure of cellulose in plant tissues by means of the polariscope, and BAILEY and KERR (**2**) have added to our knowledge of the lamellar structure of cell walls, with a series of excellent photographs taken through Nicol prisms.

It is of fundamental importance to consider the possible crystalline character of protoplasm. The polariscope has not shown protoplasm as such to be anisotropic, but it has shown striped muscle and contractile tissue in general to be so (*i.e.,* not having the same properties in all directions, as is true of crystals).

Microdissection

Microdissection and microinjection, with all forms of micromanipulation, are now included in the term *micrurgy.* The method, like all scientific discoveries, developed from primitive manipulative efforts on the part of pioneer workers. The first satisfactory instruments were constructed by BARBER (**3**) and by SCHOUTEN (**23**). BARBER built his pipette holder for the purpose of isolating single bacteria from cultures. The instrument is now replaced by several other types, each of which has its own peculiar advantages. The best known instruments which are commercially obtainable are the ZEISS-PÉTERFI (**21**) and the LEITZ-CHAMBERS (**6**) instruments. The TAYLOR (**31**) micromanipulator, privately manufactured, was an early design built for stability. A recent commercial model is that of FITZ (**8**), manufactured by Bausch and Lomb. A new but as yet little known model is that of DE FONBRUNE (**37**). It promises greatly to facilitate microdissection technique. It consists of a universal joint which controls three pistons operating against air. By means of three tubes, the pressure exerted by

the pistons is conveyed to a separate machine where three metal diaphragms, similar to those of aneroid barometers, are forced out or drawn in, and thus control, by means of levers, the rod to which the microneedle or pipette is clamped. The manipulator operates with remarkable precision, being wholly free from lost motion. The independence of operating and receiving mechanisms eliminates vibration. The hand of the operator and the needle-point move in the same direction and the operator's hand need never leave the one lever with which all movements are performed. DE FONBRUNE has also constructed an instrument for automatically drawing needles and pipettes under the microscope lens. In addition he has devised a simple method for protecting preparations of living material from dehydration without the use of a moist chamber. He simply places the material under an oil drop (nujol) either on the upper side of a slide or as a hanging drop. The oil is harmless, and lack of air has no ill effect for some time. Unfortunately the DE FONBRUNE micromanipulator is not yet commercially obtainable.

It is impossible to say which instrument is the best. The last mentioned gives promise of being so, but it has not yet been used by anyone except its inventor. Among the others, it is primarily a question of the instrument upon which one has learned. The LEITZ-CHAMBERS instrument is stable; the ZEISS-PÉTERFI has the greatest freedom of movement.

Accessories to micromanipulators are many. The fewer bought the better (certain ones sold are worthless). Exceptions are the needle-holders of Zeiss and the micropipette equipment of Leitz. Needles are best made by the individual investigator, because the type of needle should fit the work to be done, and the various types can be made only by hand. However, automatic devices such as drawing needles and pipettes are to be had (manufactured by E. Leitz). Microscalpel, microcauter, micromagnet, microelectrode, and microthermocouple are some of the appurtenances to micromanipulation. The construction of each requires a special technique.

The problems of micrurgy are many, and some have been quite successfully attacked. Certain of them are of interest not only in themselves, but have important bearings on related problems, thus the elastic quality of protoplasm, ascertained by micromanipulative methods, is of value as an indicator (the best we have) of the structure and fundamental nature of protoplasm. Other problems which remain untouched, or as yet unsolved by this method, are the structure of the chloroplast and of other plastids (is the chloroplast a droplet or a sac?), the removal of a chloroplast from one cell and its injection into another cell, the reality and nature of spindle fibers, the physical properties of the huge, motile, male gametes of cycads, and the eternal problem of the outer protoplasmic membrane and the tonoplast which is not yet fully elucidated to the satisfaction of all.

Electrical properties

The determination of electrical forces in organisms and cells is a fascinating study which is as yet in its infancy. These forces reach as high as a thousand volts, as, for example, in the ray-fish or electric eel, while that existing between the parts of plants, or between one cell and another, is of the order of 50 millivolts (0.050 volt). It is toward the measurement of these small electrical forces that our attention is here directed. This involves the use of microelectrodes (**15**) and a potentiometer circuit with amplifiers for greatest accuracy. Circuits for such measurements have been used and described by LUND (**15**) and his coworkers in the determination of potentials in trees.

Electrical investigations on cells and protoplasm require microelectrodes controlled by micromanipulative methods. Metal electrodes, even when of platinum, are likely to cause disturbances within the protoplasm; agar electrodes (agar with salt solution in a micropipette) are to be preferred. While in this review of methods we are concerned only with apparatus, it should be said that extensive work on other forms of potentials, namely, oxidation-reduction potentials, has been done with the aid of indicator dyes such as are used for pH determinations.

Cataphoresis studies have yielded some fundamental results on the electrical properties of living cells. The method involves subjecting suspensions of cells (or colloidal particles) to a difference in external potential; that is to say, the cells are placed in an electric field, and as all suspended particles, whether of metallic gold, oil, or living cells, possess an electric charge (with few exceptions), and, if free to move, they will migrate. The direction of migration is toward the pole of opposite sign to that of the charge on the particle. The rate is proportional to a number of factors including the potential of the external field and the potential (the interfacial potential) of the particles. These factors are all taken care of in the HELMHOLTZ formula for cataphoretic migration (**37**). With as yet no convincing evidence to the contrary, it can be definitely stated that all living cells are negatively charged when suspended in a solution similar (in regard to salt concentration and pH) to that in which the cells normally live. The interfacial potential of colloidal particles and cells in suspension is of the order of 30 to 50 millivolts; settling out (for example, of bacteria) occurs at about 11 millivolts.

The apparatus so far most extensively used in America for cataphoretic work is the NORTHROP-KUNITZ (**20**) model. It consists of a very shallow glass chamber open at the two ends to receive the electrolytic solution which connects the chamber with two poles of zinc immersed in zinc sulphate. A more recent model is that of ABRAMSON (**1**) which is less expensive and has the advantage of rigidity as it is constructed of one piece. The

NORTHROP-KUNITZ model is commercially obtainable. The ABRAMSON model is as yet only privately made.

To make simple and relatively crude cataphoretic observations is an easy task, but to make accurate measurements is quite another matter. The beginner is cautioned against the use of simple types of cataphoretic chambers which have been offered for sale. They are well-nigh useless and the results obtained with them are wholly unreliable. Practically all work purporting to show that cells are positively charged is due to the use of such, or home-made apparatus. Failure to measure pH is sometimes responsible for the trouble, for the sign of the charge on many suspended particles can be reversed by a change in the pH of the solution.

Simple comparative and reasonably accurate experiments in cataphoresis can be performed without calibrating the instrument, but for exact work this must be done. The best method for doing so is that of ABRAMSON (**1**).

Work in cataphoresis has yielded some of the most interesting and possibly far-reaching results of recent experimentation on the physical properties of cells. Only brief reference will be made to this work here, merely to call the attention of the student to articles wherein technique as well as results are described. Some of the earliest research on the cataphoretic migration of colloidal particles and bacteria was done by FREUNDLICH (**9**); then followed work by NORTHROP and KUNITZ (**20**) establishing the *critical* potential of bacteria. FALK (**7**) correlated the pathogenicity of bacteria with their potential. (This work may prove in part erroneous, but it was highly suggestive, pointed the way, and may yet be right to a degree.) More recent is the important work of MUDD (**17**) on sperms and bacteria, of ABRAMSON (**1**) on protein-covered quartz particles, and of MOYER (**16**) on latex. The last-mentioned investigation has made a fundamental contribution by showing that kinship or species relationship of Euphorbias is related to the shape of the mobility curves, and, primarily, to the iso-electric points of the latex particles.

The centrifuge

The centrifuge has played a part in studies on protoplasm chiefly in regard to determinations of viscosity. The first work of this kind was done by NĚMEC (**18**). He found that changes in the viscosity of protoplasm occur during cell division. In the young dividing cells of the onion, bean, etc., resting nuclei are, by centrifuging, thrown against the apocentric cell wall, while spiremes and asters remain situated in the center of the cell. Metakinetic and telophasic figures are only slightly displaced. The most easily disturbed stages of cell division are those coincident with the formation of the cell wall. In other words, the protoplasm of the resting cell

preparatory to division is of low consistency; the protoplasm of the cell in mid-mitosis and immediately following is of high consistency, and the protoplasm of the cell in the last stages of division is of minimum consistency.

Recent developments have resulted in the construction of a small air-driven centrifuge which attains the highest speed yet known for centrifuges (**5**).

Centrifuging is but one of several ways of determining protoplasmic consistency. Other methods are microdissection (**24**), magnetic properties (**37**), the fall of statoliths (**37**), plasmolysis (**34**), and Brownian movement (**4**). Misunderstandings which have arisen, and a convincing clarification of them, have recently been set forth by FRY (**11**); he employed the centrifuge method.

All determinations of protoplasmic consistency by whatever method involve a consideration of the important question of the applicability of the single constant laws of Newton, Poiseuille, *et al.*, developed for true solutions and pure liquids, to systems such as protoplasm, the behavior of which is anomalous. The problem is an important one and involves recent rheological theories on non-Newtonian fluids (**37**). Investigations on the viscosity of protoplasm are many and the bibliography extensive; these have been reviewed by WEBER (**33**) and others (**25**).

Photography

Photographs supply an authentic record of an experimental result. Photomicrography is now so highly developed a science and art that a discussion of it here would be wholly inadequate. A few remarks of interest and perhaps value may, however, be made. The small, $4\frac{1}{2} \times 6$ or 9×12 cm. camera which rests directly upon the microscope tube is very convenient, and excellent results can be obtained; but many workers still prefer to use the old-fashioned camera-box swung upon an upright support. The latter equipment is certainly the more flexible of the two, but it lacks one very convenient attachment of the former—the beam-splitter. It is a decided advantage to be able to focus through an ocular rather than upon a ground glass. Certain new but expensive photographic instruments combine the advantages of the old type camera-box and the new microscope camera with beam-splitter. The production of good photomicrographs is to a considerable degree dependent upon a correct choice of light filters. These can be had in considerable variety. The infrared filter is a recent addition and is to be used with special infrared sensitive plates. The photographer must try out the various combinations of lights, filters, and negatives suitable for his particular material.

The introduction of cinematography into microscopic work has yielded some fascinating results which are of real value to the research worker as

well as to the teacher or student. With the aid of moving pictures, details in mitosis heretofore unsuspected have been discovered. (A word of warning should be given in regard to the misleading impression created by speeding up films; pictures taken at the rate of one a second or one a minute then shown at the rate of 32 a second often give a highly entertaining but scientifically inexact impressions.) Motion pictures of tissue-cultures show the migration and division of fibroblasts and the movement of chromosomes in culture; others illustrate the development of the growing mammalian embryo from the one-celled stage to the sixteen-celled stage. Very striking are moving pictures taken with dark field.

For botanists, moving pictures have made their chief contribution in such studies as those on the mechanism of conjugation in *Spirogyra,* and on the operation of the animal-trap of *Utricularia.* These studies have been carried out by LLOYD (**13**). Through them is the method of movement of the male gamete in *Spirogyra* for the first time fully understood (motion is by contraction of vacuoles). A recent application of the cinematograph to protoplasmic studies has involved photographing protoplasmic streaming. Streaming protoplasm is always fascinating to watch, and becomes even more so when seen on the screen. But the method has a practical application; events which take place but once and too hurriedly to permit careful observation can be seen again and again if they have been photographed as moving pictures and are then shown at slow speed. The equipment necessary for taking moving pictures can be expensive but need not be. An essential feature is the beam-splitter so that material may be seen while it is being photographed. Other than this, any type of amateur camera for cinematography is all that is needed. The 8-mm. film is fairly satisfactory, but better results are to be had with the 16-mm. or professional 32-mm. film. The saving in cost of the 16-mm. over that of the 32-mm. film is to be considered in regard to the film, camera, and projector. Perhaps in no other way can an idea of the perpetual activity of protoplasm be so dramatically conveyed as through a moving picture of the streaming protoplasm of myxomycetes.

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EFFECTS OF X-RAYS ON *ZEA MAYS*

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(WITH EIGHT FIGURES)

Introduction

Since the discovery of x-rays, their effects on both plants and animals have been studied as important biological problems. Many reports of early work are conflicting because quantitative results have been possible only in recent years, after methods of accurately measuring the dosage were perfected.

In the study of many general problems, plants are ideal material because of the large numbers which can be used and the ease with which environmental conditions can be modified or kept constant as occasion demands. Samples can be taken for cytological studies as the experiment progresses without detracting from the final results. Numerous reports on plant experiments have come from hospital laboratories where the work has been done with the conviction that many of the results will be applicable to problems of animal x-ray technique.

The work reported here was started for the purpose of finding out how *Zea mays* would react to x-ray treatment in various aspects of its growth and appearance, in order to use it eventually as a test material for studying effects of irradiation as they might be influenced by changing environmental conditions. *Zea mays* has been used occasionally for special radiation problems. STADLER (**14**) found it valuable for studies of mutations produced by x-rays because its genes had been more thoroughly mapped than those of many other plants. BERSA (**2**) reported *Zea mays* to be relatively more resistant to irradiation than many plants which he tested. He used it extensively for cytological examination of irradiated root tips.

Materials and methods

The source of radiation was a standard air-cooled Coolidge tube. A constant potential of 180 kv. was used and the filament current was maintained at 3.7 ma. The distance from the center of the target to the material to be irradiated was 30 cm. The only filters were the heavy pyrex glass of the tube and the paper over the seedlings. The actual strength of the x-rays used was measured during radiation by the usual ionization-galvanometer arrangement, the ionization chamber having been calibrated previously with a standard chamber as described by TAYLOR and SINGER (**17**). The calibration of the apparatus gave a factor of 1.7 r units per centimeter of deflection of the galvanometer. Thus at a deflection of 15 cm. for 40 minutes, the dose

would be $1.7 \times 15 \times 40$ or 1020 r. The ROENTGEN unit or "r" was defined at the Second International Congress of Radiology in 1928 as follows: ". . . the quantity of radiation which, when the secondary electrons are fully utilized and the wall effect of the chamber is avoided, produces in one cubic cm. of atmospheric air at 0° C. and 76 cm. mercury pressure such a degree of conductivity that one electrostatic unit of charge is measured at saturation current" (**16**).

Improved Golden Dent field corn was used in all experiments. The dry grains were planted between layers of moist paper toweling in glass chambers placed in a dark incubator at 24° C. After 72 hours most of the primary roots were slightly more than 1 cm. long. The grain was always placed in the germinating chamber in the evening at about the same hour in order to avoid differences in sensitivity due to possible natural mitotic rhythm. These precautions were also for the purpose of obtaining an equal degree of hydration in all experiments.

Seedlings were selected which had fairly straight roots 10 to 15 mm. long. A spot of India ink was placed exactly 10 mm. from the tip of each root and all future measurements were made from this point. In most experiments 20 seedlings were used in each group. The plants to be treated were individually placed on damp filter paper over the bottoms of glass dishes. The side of the grain where the shoot was about to emerge was always placed up in order that all of the embryos might be equally exposed to the x-rays. The dishes were closely covered with three layers of moist Scott Tissue toweling to prevent evaporation from the seedlings during radiation. The controls were kept under the same conditions outside of the x-ray apparatus.

Immediately after treatment the seedlings were placed either in pots of damp earth for observation in the greenhouse or in jars of loosely packed sphagnum to be put into the incubators for root study. The irradiated plants grown in soil had the normal greenhouse conditions of light and temperature. A large number of pots were prepared at one time with the same lot of soil to insure uniformity of nutrition.

Results

EFFECT OF X-RAYS ON AERIAL PARTS OF *ZEA MAYS*

The following observations were made on a typical series raised in the greenhouse. Groups of 12 seedlings each received doses varying from 100 to 5000 r. Up to the fifth day there was no difference in the heights of the shoots of the different groups, but on the fourth day close inspection showed that there was a delay in the bursting of the sheath or coleoptile in the heavily irradiated plants. The number in each group with the shoots visible at this time was as follows: controls, 11; 100 r, 7; 200 r, 8; 1200 r, 1; 1600 r and above, 0. On the sixth day all of the controls, 100, and 200 r groups had

burst the sheath as had also about 50 per cent. of the plants of the more heavily irradiated groups.

Measurements made 8 days after irradiation showed that all growth of the sheath had stopped both in the control and in all of the treated groups. The following figures indicate that the net growth of the sheath was not retarded by irradiation: average height of control, 22 mm.; irradiated with 800 r, 25 mm.; and 2000 r, 23 mm.

From the sixth day, the growth in height of the plants receiving doses of 1200 r and higher fell behind that of the controls. The upper limit of the amount of radiation which allows the majority of seedlings to unfold the first leaf was about 1200 r. When a dose of 800 r was applied, the growth rate during the first month was equal to that of the controls when only the height of the shoots was considered. When part of this 800 r group was removed from the soil and measurements made 12 days after irradiation, the roots had an average length of 207 mm. as compared with 238 mm. for the controls. After three months the tops of the remainder of the 800 r group were about two-thirds of the height of the controls. This may mean that in the case of the shoots there was a very long latent period before the effects of irradiation could be observed. On the other hand, it is possible that this later dwarfing was caused by lack of root development owing to x-ray injury, rather than a direct effect of irradiation on the tops themselves. After a dose of 1200 r, most of the seedlings died in two weeks, having grown little more than the group which received 2000 r, while the survivors, a quarter of the whole number, grew as well as the plants in the 800 r groups. At a dose of 1200 r the individual sensitivity seemed most apparent. The individuals in the groups receiving doses between 1400 and 5000 r showed little variation, either in length of roots, which averaged 54 mm. at death, or in height of shoots, which averaged 45 mm. at that time.

The leaves of plants which received a dose of 200 r and more showed many small areas of yellowish green between patches of normal color. Up to 400 r, the first three leaves were the only ones showing this chlorotic disturbance. With higher doses, which still allowed fairly normal growth of tops, a much longer time elapsed before normally colored leaves were produced. With very high doses the occasional plants which were able to produce one leaf, were decidedly yellow with very small areas of normal green color.

Leaves in the 800 r group, which were of normal length, showed a peculiar twisting and curling in some cases. The edges of the leaf were slightly inrolled and tended to move toward the under side. This condition persisted throughout the three months of the experiment.

Special root studies

The seedlings grown in sphagnum were used for observations of the behavior of roots after irradiation. Tests showed that the plants could be

carefully removed from the jars twice a day without affecting the rate of growth. It can be seen from table I that the final length attained by roots

TABLE I

ROOT LENGTH ATTAINED IN SPHAGNUM AFTER KILLING DOSES OF X-RAYS

TEMPERATURE	DOSE IN R UNITS	HOURS AFTER TREATMENT	AV. LENGTH
°C.		hr.	mm.
22	10,000	77	32
"	7,000	77	28
"	5,000	77	31
"	5,000	63	34
"	4,000	63	31
"	3,000	60	43
29	3,000	50	41
24	2,500	68	53
17–21	2,000	134	51
22	2,000	90	52
24	2,000	70	50
24	2,000	94	62
24	2,000	85	51
29	2,000	40	52
35	2,000	45	44
36	2,000	42	47
36	2,000	43	41

after receiving doses of 4000 to 10,000 r shows great uniformity. The growth curves are also practically identical throughout this range. A dose of 4000 r would then appear to be near the limit beyond which further irradiation could be expected to have no observable effect, at least under these experimental conditions. As the dose is reduced below 4000 r there is a fairly regular increase in the rate of growth of the primary root after irradiation. Doses below 150 r produce no measurable differences between such treated seedlings and their controls when primary roots alone are tested.

A study was made to determine the smallest dose that would consistently produce a measurable difference between such treated seedlings and their controls. Preliminary observations showed that the rootlets growing laterally from the primary roots are the parts of the plant which first exhibit visible evidence of x-ray injury, although these rootlets cannot be seen at the time of irradiation. It has been found (table III) that, in all experiments at a given temperature, the time required for lateral roots to appear on normal seedling is remarkably constant. Table II shows that the time between irradiation and the first appearance of lateral roots steadily increases with the dose from 75 to 1500 r, which is near the upper limit of the amount

TABLE II

TIME OF APPEARANCE AND POSITION OF LATERAL ROOTS WHEN GROWN AT 24° C. AFTER X-RAY TREATMENT

DOSE IN R UNITS	APPEARANCE IN AT LEAST 33% OF CASES			AV. LENGTH OF PRIMARY ROOT	AV. DISTANCE OF FIRST ROOTLET FROM SEED
I	TREATED	CONTROLS	DIFFERENCE	V	VI
	II	III	IV		
	hr.	*hr.*		*mm.*	*mm.*
60	61	61	0	94	0
75	84	68	16	116	0
150	93	68	25	103	0
300	100	68	32	113	.....
300	87	63	24	122	6
600	94	67	27	98	.....
600	96	67	29	110	13
1,000	90	64	26	90	.....
1,000	114	63	51	65	25
1,000	160	68	92	98	20
1,500	160	67	93	65	31
1,500	160	68	92	73	20

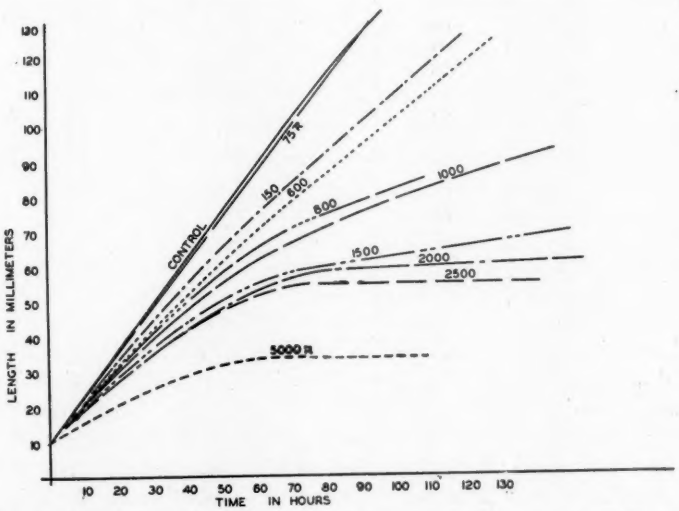

FIG. 1. Effect of various doses of x-ray on growth of primary roots of *Zea mays* in sphagnum at 24° C.

of irradiation that allows a few lateral roots to be formed on most of the seedlings.

Figure 1 shows that the growth rate of the primary roots treated with 75 r was identical with that of the controls, but the lateral roots in this irradiated group appeared 16 hours later than those of the controls. It has been stated above that 150 r was the smallest dose that could be depended upon to check the growth of the primary root in most of the corn seedlings.

Control seedlings grew at an increasing rate as the temperature was raised from 17° to 36° C. In these plants the lateral roots tended to emerge when the primary root was about 100 mm. long, irrespective of the time required to reach that length at various temperatures. Table III shows that

TABLE III

TIME OF APPEARANCE OF ROOTLETS ON CONTROLS AT DIFFERENT TEMPERATURES

TEMPERATURE	TIME OF APPEARANCE OF ROOTLETS		AV. LENGTH OF MAIN ROOTS	
°C.	*hr.*	*hr.* (mean)	*mm.*	*mm.* (mean)
37–39	39	39	112	112
36	38		106	
36	36	37	82	94
35	44		73	
35	40		129	
35	36	40	115	104
29	40		95	
29	36		91	
29	43		104	
29	39		108	
29	47		104	
29	45	42	96	98
24	63		90	
24	68		99	
24	67	66	119	103
22–24	96		122	
22	97	96.5	122	122

with increasing temperatures the time required for the appearance of lateral roots decreases, while the length of the primary root at this time is very constant. For instance, in a group raised at 22° C., after the usual germination period at 24° C., the lateral roots appeared 97 hours after the first measurement. In another group raised at 35° C. the rootlets appeared after 48 hours, the average length of the primary roots being the same in each case. Figure 2 shows the very regular rate of decrease in time necessary for the appearance of rootlets as the temperature increases.

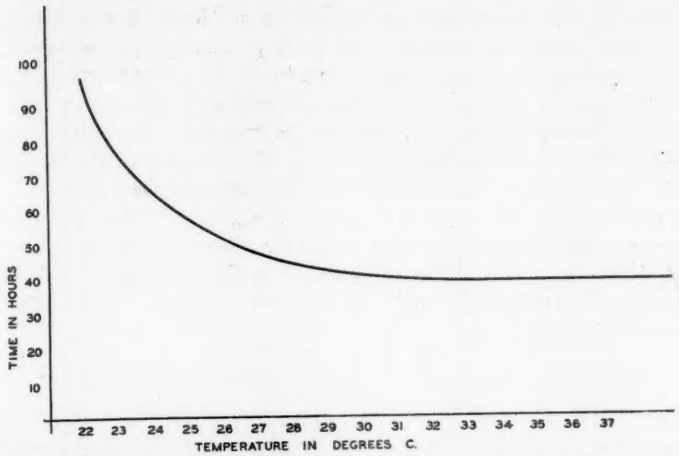

FIG. 2. Effect of increase in temperature on time required for appearance of secondary roots on seedlings of *Zea mays*.

Careful examination of the control seedlings showed that the rootlets first appeared on the primary root immediately below the grain in all cases, no matter how long their appearance was delayed by cold and the slow growth

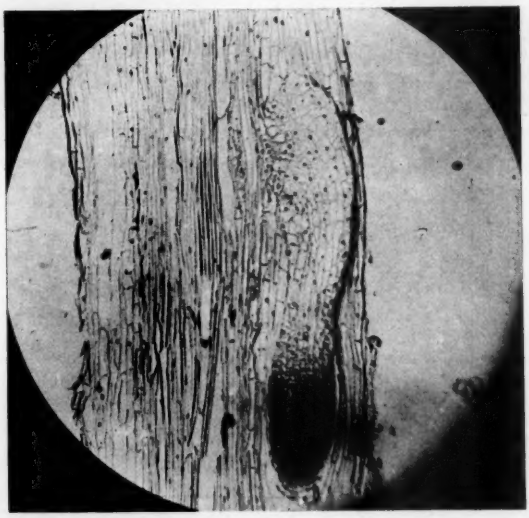

FIG. 3. Section showing abnormal growth of secondary root after an x-ray dose of 1500 r.

accompanying it. It can be seen from column VI of table II that, when doses of 300 r and more were used, the average distance of the first lateral root from the grain increased from 6 mm. to 31 mm. after a dose of 1500 r. The roots grown in soil showed this same type of behavior after irradiation. With the higher doses the primary root had only a tassel of rootlets very close to its tip. Histological sections made from roots which had received a dose of 1500 r showed primordia of lateral roots in the region just above the level where rootlets were growing out in a normal manner. In some cases these primordial rootlets were subnormal in size and in a few examples they had turned either up or down, growing parallel to the longitudinal axis of the primary root, and they were entirely inclosed within its tissue. Figure 3 illustrates this condition.

Effect of temperature on growth after x-ray treatment

Experiments performed to test the effect produced by keeping the corn seedlings at 6° C. during irradiation with doses of 200 to 1000 r and then allowing them to grow at 24° C. showed no differences between such treated seedlings and those similarly irradiated at room temperature. Following the failure to produce differences in sensitivity to x-radiation by means of low temperature during treatment, a number of tests were made to find out whether varying the temperature after x-ray treatment would affect the amount of injury due to irradiation. Since corn was found to thrive at temperatures of 17° to 36° C., it was possible to observe the behavior of roots whose growth rates varied widely according to the temperature selected. In the first experiment three groups of seedlings were irradiated at the same time with 2000 r, and then the separate lots were placed, with their controls, in three incubators at varying temperatures. Care was taken to measure the growth at very frequent intervals. Figure 4 shows the curves made by the

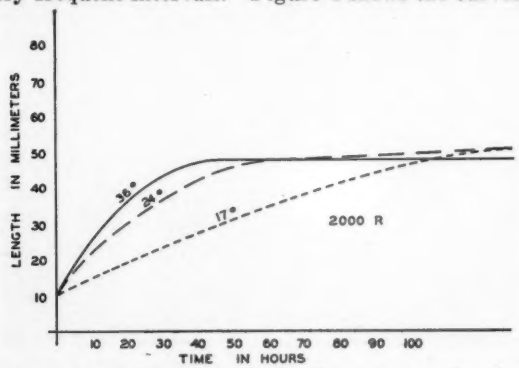

Fig. 4. Curves of growth at three temperatures after equal amounts of radiation. Length of life increases as temperature decreases.

growth of the primary roots of these seedlings exposed to 36°, 24°, and 17–21° C. after all were irradiated under exactly the same conditions. The shape of the curves is very significant, showing that the time of death was

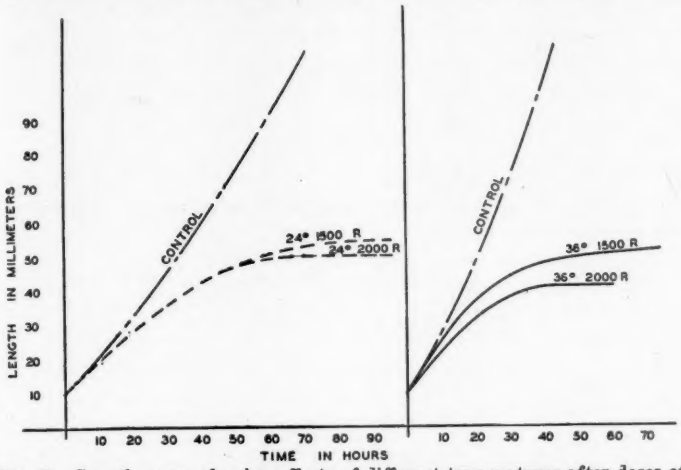

FIG. 5. Growth curves showing effects of different temperatures after doses of 1500 and 2000 r. Increasing doses decrease the latent period and the effect is exaggerated by increase in temperature.

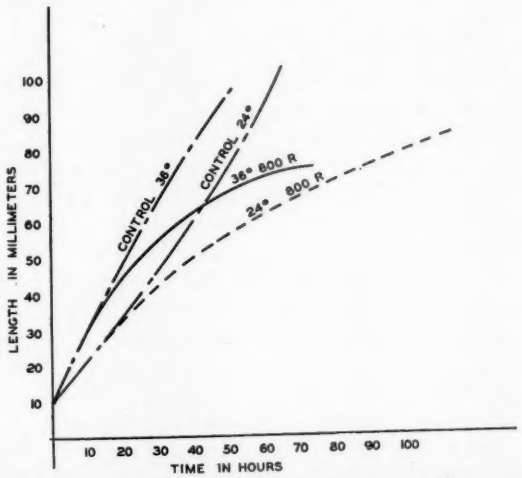

FIG. 6. Growth curves of primary roots of *Zea mays* showing effect of exposure to 24° and 36° C. after a dose of 800 r. At 20 hours the irradiated group at 24° C. shows no injury while at 36° C. roots are 4 mm. shorter than their controls.

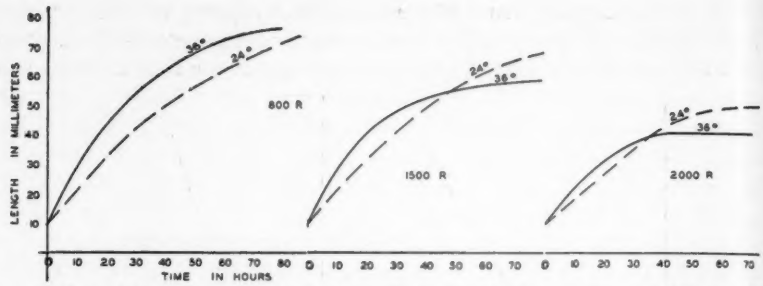

FIG. 7. Curves showing comparative effects of exposure to 24° and 36° C. after exposure to various doses of x-rays.

delayed as the temperature decreased. Similar experiments were performed by repeating the dose of 2000 r and also using 1500 and 800 r, in each case exposing part of the irradiated group to 24° and part to 36° C. with their respective controls at each temperature. Figure 5 shows that after both the 1500 and 2000 r treatments, the roots grown at 24° lived longer than those in the group at 36° C. Growth after 1500 r extends over a longer period of time at the lower temperature, and, also, the roots reach a considerably greater length before death when grown at 24° than at 36° C. Figure 6 shows that when the dose is lowered to 800 r, the irradiated groups grown at

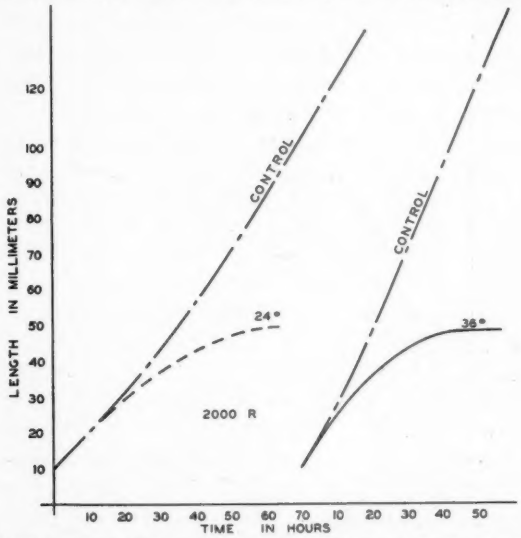

FIG. 8. Growth curves showing effect of temperature on length of latent period after an x-ray dose of 2000 r.

the two temperatures grow roughly parallel to each other, the 36° group growing the faster just as the 36° controls grow faster than the 24° controls. In figure 7 the curves of the growth following the three doses have been rearranged to show more graphically the effect of the two temperatures on each. The curves in figures 4, 5, 6, and 8 show that with all the doses used, the latent period was longer at the lower temperature. As the dose increases, the difference between the temperature effects is increased.

Discussion

The coleoptile was the only part of the corn seedling which failed to show any effects of irradiation. CATTELL (**3**) reported it to be the least sensitive part of wheat, as after a dose of 1200 r the coleoptiles showed a reduction of 8 per cent. in height while the primary roots were reduced to 45 per cent. of the length of the controls. It would appear that the coleoptile of corn is less sensitive to irradiation than the coleoptile of wheat. The apparent lack of sensitivity to x-rays of this organ may be partly explained by the results of cytological studies of *Zea mays* by TETLEY and PRIESTLEY (**18**). They found that cell division stops in the coleoptile earlier than in any other part of the embryo. The fact that cell division has probably ceased long before exposure to x-rays may account for this organ showing no injury after doses which seriously interfere with the growth of parts of the seedling in which there has been rapid cell division at the time of irradiation.

SKOOG (**13**), using moving pictures to study the behavior of the coleoptile in *Avena*, found that its growth stopped when it was ruptured by the shoot pushing through it. If it is natural for the growth to continue only as long as the sheath is intact, and if the rupture is delayed when the shoot is stunted by irradiation, then this tendency towards excess growth in height may mask the effect of x-ray treatment on the corn coleoptile. The fact that the coleoptiles of treated corn seedlings were a trifle longer than those of the controls may indicate that the stunting effect of x-rays was a bit less influential than the opposite tendency towards increased growth.

The chlorotic disturbance noted in the corn leaves is typical of the results reported for many species. NOGUCHI (**10**) examined sections of leaves from irradiated seedlings of *Helianthus annuus* and found that the light color was due both to a reduction in the number of chloroplasts and a deficiency in their chlorophyll content.

The fact that irradiation in any amount between 4000 to 10,000 r failed to produce more stunting of the primary root than did 4000 r, shows that corn responds to x-rays in this respect in very much the same manner as does wheat. FRANCIS (**5**) reported that doses increasing from 3390 to 13,000 r fail to produce any increase in damage to the seedlings either in affecting the growth or in reducing the rate of respiration. Many workers have tried in vain to find a dose of x-rays strong enough to kill the plant immediately.

In an early experiment, where it was necessary to compare the effects of irradiation on two groups of seedlings which had been treated under different conditions, the time for comparison was chosen when both groups had lateral roots starting but those of one set were further developed than those of the other. Quantitative measurements were made at first by carefully cutting off the rootlets, obtaining the dry weights of those of the two groups, and then computing the average weight of the lateral roots of each group. This laborious method was discarded when further study showed that small differences in the amount of damage caused by irradiation could be measured both by noting the time at which lateral roots appeared and also by recording the distance below the grain at which the first rootlets appeared. The use of the time factor is the more exact of the two methods. It is necessary to decide on a given proportion, such as one-third, of the plants to be required to show rootlets before the time of appearance is recorded. This helps to prevent mistakes being made when the group contains one or more freak plants which, for some unknown reason, are unusually resistant. Such individuals have been omitted in computing averages when they were few in number and extremely different from most of the group.

When preliminary experiments have been carried out at the same temperature and near the dose to be used, the time interval during which very frequent observations are necessary to establish the time of appearance of rootlets can be fairly accurately predicted. In order to compare the sensitivity of two groups of corn seedlings, a dose of 300 r would have the advantage of yielding data both on the time of appearance and the spacing of lateral roots below the grain, and it would furnish this information in a shorter time than would be required for the same observations after higher doses. WIGODER and PATTEN (**19**) observed that lateral roots of *Vicia faba* were delayed in the time of their appearance and were formed more and more distally from the seed as the dose of x-rays was increased. It is therefore possible that this type of reaction could be expected in many kinds of plants and could be used as a general method for comparing sensitivity to irradiation between groups of the same species.

The fact that the appearance of the first lateral roots is delayed by irradiation might indicate that time is required for the repair of certain damage before these roots can be formed in a normal way. The few sections which were prepared from seedlings which had been irradiated with 1500 r showed no indications of lateral roots being formed in the region just below the seed, while in the region further down there were abortive attempts at lateral root formation followed by a region producing apparently normal rootlets. Since irradiation is generally considered to have an aging effect on tissues, the failure of the lateral roots to reach the exterior along the upper part of the root might be due to the same type of conditions which prevent secondary

root formation in old, tough roots. Further study may determine whether the rootlets in the x-rayed material changed the direction of their growth because of the mechanical difficulty of penetrating the outer layers of the primary root.

In normal seedlings there was a quite definite relation between the length of the primary roots and the time of appearance of lateral roots, but this relationship was upset in the irradiated groups. Here the rootlets tended to appear while the primary root was relatively shorter, but if the plant's main root never reached a length of 60–70 mm. it was unlikely that any rootlets would be formed. It may be possible that as the plant begins to suffer from the slow growth of the x-rayed primary root, the lateral roots tend to appear earlier in proportion to the length of the main root as an attempt at compensation for the lack of absorbing power of the main root. JOHNSON (7) reported that irradiated seedlings of *Helianthus annuus* lack ability to absorb as much water as their controls. This condition is all the more serious for the plant, because the aerial parts, not being stunted by irradiation to the same extent as the roots, are in need of a supply of water approaching that of a normal plant.

There are reports of several experiments on the effect of irradiating living material at low temperatures. CRABTREE and CRAMER (4) treated mouse tumor with radium at 0° C. and found that its sensitivity to irradiation was greater than when it was treated at room temperature. MOTTRAM (9) obtained similar results, using *Vicia faba* (a very small number of plants) treated with both radium and x-rays. On the other hand, HENSHAW and FRANCIS (6) failed to find any difference in the sensitivity of wheat seedlings when irradiated at different temperatures. The same type of experiment was tried in this laboratory with corn, with the idea that this plant, being more sensitive to cold than wheat, might give somewhat different results. The findings were negative.

It was found that growing corn seedlings at different temperatures following irradiation produced differences in the length of the latent period, the ones grown at the higher temperature showing the damaging effects sooner than those which were allowed to grow more slowly at the lower temperature. This held true for groups of roots which had received a dose of 800 r and grew in a manner very nearly like the normal. The effect on the latent period became more and more noticeable as the dose was increased to 2000 r. It would be of interest to test higher doses to determine whether a stage could be reached where the damage done by irradiation would be so great that a comparatively low temperature could have no effect towards lessening the amount of injury.

With all biological material the time required for the effects of irradiation to appear varies according to the dosage, the type of organism, and probably

many other factors. It is generally considered that biological changes are the result of chemical changes set in motion by ionization during the actual time of irradiation. Conditions which would speed up or modify the chemical reactions could be expected to shorten the latent period required for the results of the biological changes to appear. Heat increases the speed of most chemical reactions and does appear to shorten the latent period in the experiments reported here.

Although irradiated seedlings grown at 36° C. begin to show the effects of irradiation at an early age, they grow fairly rapidly until they suddenly die. When groups of irradiated plants grown at successively lower temperatures are compared, it appears that the slower the plants grow, the longer they are likely to live. However, the dose of 2000 r has set a limit, perhaps within a range of 6 mm., to the amount of growth possible following that amount of irradiation. The seedlings grown at each temperature group die when their primary roots have reached this limit no matter how long a time is required for each group to grow roots of that length. This limit may be determined by any one of several factors or a combination of them. A given amount of food material may be available for the building of new root tissue and irradiation may prevent the formation or transportation of more of that substance after the original supply is exhausted. If the root is growing rapidly, it will use up its supply and die sooner than a slow-growing root which uses up its food more slowly. This theory might help explain why the primary root can grow to the same length, when either 4000 or 10,000 r have been applied. If there is the same amount of food material available originally for both groups of roots, the 4000 r dose is just as effective as the 10,000 r dose in preventing any further growth after the original supply is exhausted. As the dose was reduced below 4000 r this food formation was apparently less and less disturbed. Since growth of the plant as a whole proceeds quite normally after a dose of 800 r, it may be assumed that there is early recovery if this amount of irradiation interferes with the food supply.

Another cause of death may be the accumulation of mildly toxic substances until they interfere with natural physiologic processes, or, as SCHWARTZ (**12**) suggests, the proteins may be giving rise to increasingly toxic products as their decomposition proceeds. Either of these conditions would be aggravated and cause earlier death with increase in temperature and the higher rate of metabolism accompanying it. The difference between the length of the latent period at different temperatures in the case of plants receiving 800 r, might be due to the difference in the rate at which toxic materials were formed. It is possible that some of these poisonous substances may diffuse out; this would help explain part of the recovery which takes place when the doses of radiation are not too heavy.

The comparative lack of irradiation injury shown in the early stages by plants grown at 24° C. and below might be due to the time element of delay

made possible by the use of lower temperatures. Several authors have tested the effect of allowing time to elapse between irradiation and the beginning of growth processes following it. STRANGEWAYS and FELL (15) reported that when chick embryos were kept in the refrigerator for 24 hours after irradiation and then allowed to grow in incubators at their normal temperature, the injury due to irradiation was less than the injury suffered by embryos similarly irradiated but allowed to continue their normal growth rate immediately following exposure to x-rays. There may be a certain amount of recovery going on during the period before growth is resumed.

After living material has been irradiated there is a certain amount of residual effect which continues at a steadily decreasing rate. According to KINGERY (8), the rate of decrease of this effect with time follows a logarithmic curve. If growth processes are delayed for a time, the amount of residual effect will be growing less and less in the meantime. Therefore, when the rate of metabolism is allowed to return to normal there will be less damage done to the cells which are again beginning to divide than would occur if they continued dividing immediately after the irradiation period. In like manner, the long, slow growth made possible by low temperature could be assumed to reduce radiation injury by allowing more time for the reduction of the amount of residual radiation.

PACKARD (11) performed a set of experiments on Drosophila eggs which very nearly parallel the tests reported here on growing corn seedlings at different temperatures following irradiation. After the same dose of x-rays, groups of eggs which were incubated at 18° C. hatched in 80 per cent. of the cases, while in the group kept at 28° C. only 53 per cent. hatched. Low temperatures after irradiation also reduced the amount of injury shown by chick embryos in experiments reported by ANCEL and VINTEMBERGER (1).

The fact that corn seedlings, Drosophila eggs, and chick embryos all react to temperature effects after irradiation in the same general way would seem to indicate that temperature during this period controls one or more fundamental conditions involved in determining the amount of irradiation injury shown by the living organism.

Summary

Seedlings of field corn were x-rayed with doses of 60 to 10,000 r and their subsequent growth observed under varying conditions. The results are as follows:

1. The height of the coleoptile was not affected by doses up to 5000 r which was the highest dose used in these particular experiments.

2. Shoots of plants which received over 800 r showed the effects of irradiation by delay in bursting through the coleoptile and by failure to reach the height attained by their controls during the time of the experiment.

3. All of the greenhouse plants irradiated with 200 r and more showed a chlorotic disturbance in the early leaves.

4. Leaves of all plants which received 800 r, and the small number of survivors from the 1200 r group, showed a strong tendency to curl their edges toward the under surface.

5. The rootlets growing laterally from the primary root were the most sensitive part of the seedlings. Doses of 75 r and higher delayed the appearance of these secondary roots increasingly as the dose was raised, while doses of 300 r and higher increased the distance between the grain and the first of these roots. Comparison of the time of appearance and spacing of these secondary roots is suggested as a method for comparing the degree of sensitivity to x-rays of various groups of seedlings.

6. Doses up to 10,000 r failed to prevent at least 20 mm. of root growth after radiation was applied.

7. Plants irradiated with doses between 800 and 2000 r showed a much shorter latent period before x-ray effects were measurable when grown at 36° C. after treatment than when grown at 24° C. and lower temperatures.

8. After a dose of 800 r corn seedlings showed the same amount of injury whether they were grown at 24° or 36° C. After 1500 r the 24° group grew faster than the 36° group after 50 hours, and the roots attained considerably greater length during their longer life. After doses of 2000 r the 24° groups surpassed the 36° groups after 35 hours and lived longer, but after this dose the final length was never more than 9 mm. greater than that of the 36° group. On the whole, lower temperatures after irradiation tended to reduce the amount of radiation injury.

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LAG IN WATER ABSORPTION BY PLANTS IN WATER CULTURE WITH RESPECT TO CHANGES IN WIND[1]

J. Dean Wilson and Burton E. Livingston

(WITH THREE FIGURES)

Introduction

This paper presents a portion of the results of a series of wind-tunnel tests on water absorption, carried out in the laboratory of Plant Physiology of the Johns Hopkins University in 1924. We aimed to continue that study with more adequate control of air conditions and with other improvements in technique, but opportunity to realize that aim has not become available.

To make sure that the supply of water to the roots of our plants would always be unlimited, we employed solution cultures, which were usually supplied with Shive's well-known 3-salt solution of KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$, in osmotic proportions of 5 : 2 : 3 and with a total osmotic value of 1.5 atm. Rates of water absorption and transpiration must consequently have been generally limited or controlled only by internal characteristics of the plants themselves (absorbing, conducting, and transpiring capacity) and by the current evaporating power of the aerial surroundings. Excepting in some special tests, these rates were never retarded by inadequacy of the external water supply at the absorbing surfaces nor by external suction, capillarity, or osmotic resistance to absorption by the root systems. Wilting of the leaves which could be induced by exposure to wind velocity of 12–15 mi/hr., with water-vapor-pressure deficit of about 15 mm. and air temperature of 28°–33° C., indicated simply that the absorbing and conducting capacities of the plant, taken together, were inadequate to deliver water to the foliage as rapidly as water was being lost through transpiration. Several species of plants rooted in well-watered soil also showed this type of wilting and Livingston has observed (5) plants of *Fagopyrum esculentum* L. in water culture to attain what must be regarded as "permanent wilting" in a sunny greenhouse, even without notable wind.

We were not now concerned with wilting, however, nor even with water absorption and transpiration under the influence of long-continued wind. In the experiments now considered the plant was subjected to artificial wind only long enough to attain a steady rate of absorption, after which the wind was discontinued and the absorption rate for calm air was soon regained.

[1] Botanical contribution from the Johns Hopkins University, no. 136.

Methods

Rooted cuttings were employed, mostly of *Salix purpurea* L. (basket willow). *Salix nigra* Marsh (black willow) and *Cephalanthus occidentalis* L. (button bush) were used in some tests. Basket willow is especially well suited to the preparation of a large number of closely similar rooted cuttings. Cuttings were made in late January, from shoots having a basal diameter of about 1 cm. They were usually 30–35 cm. long, but some were 120 cm. in length. The basal end of each cutting, protected by a suitable piece of tapered brass tubing, was thrust through the central hole of a large 3-hole rubber stopper, which seized the stem firmly in an air-tight joint. About 15 cm. projected on the lower side of the stopper. After removing the metal tubes, the cuttings were planted in moist sand in the greenhouse, to root and sprout. About 4 weeks later those which had developed satisfactorily were transferred to sheet-metal tanks (about 120 cm. long, 60 cm. wide, and 20 cm. deep) containing nutrient solution. The tanks, which stood in the greenhouse, were each provided with a sheet-metal lid with many perforations into which the rubber stoppers fitted, so as to keep the plants upright with their root systems in solution and their developing shoots and foliage in air. At the end of an additional 4-week period the plants were well developed and ready for use.

FIG. 1. Plant-culture bottle assembly.

When an experiment was to be started, a suitable plant was transferred from the tank to a large-mouthed bottle completely filled with about a liter of nutrient solution, a glass tube was tightly set into each of the unoccupied holes in the attached rubber stopper and the latter was firmly set into the bottle mouth, as shown diagrammatically in figure 1. Tube B could be closed at will by means of a bit of rubber tubing and a clamp of the Hoffman type. The assembly was then introduced into the wind tunnel and a potometer (shown diagrammatically in figure 2) was connected thereto by means of thick-walled rubber tubing, which joined tube C of figure 1 to one of the D tubes of figure 2. The culture bottles were kept closed for periods

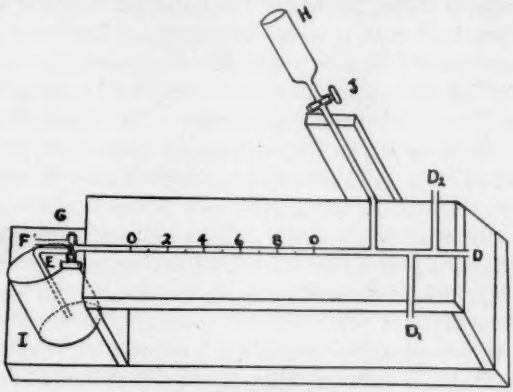

FIG. 2. Potometer.

no longer than were actually required for obtaining satisfactory potometer readings. Stoppers were lifted and allowed to rest on the bottle rims as much as possible and plants were regularly returned to the well-aerated stock tanks between experiments.

Standardized white spherical porous-porcelain atmometers of the LIVINGSTON form (7) were operated in comparison with the plants. The sphere was mounted on a vertical glass supply tube in the ordinary way, without a non-absorbing valve. The tube bore a 3-hole rubber stopper like those used on the cuttings, and the atmometer and its tube formed part of a bottle assembly similar to the one shown in figure 1. For atmometers, of course, distilled water replaced the nutrient solution. Each atmometer reading was reduced to the LIVINGSTON standard for this type of sphere by mutiplying by the operating coefficient of the sphere used, which was 0.80 in every case.

Care was necessary to avoid the presence of undissolved gas anywhere in the whole bottle-potometer system, excepting the bubble index of the

potometer. Rates of removal of liquid from the culture bottle or atmometer bottle were measured, by 0.1-ml. or 0.50-ml. increments, by means of a stop watch. These rates were first recorded in terms of the number of seconds or minutes required for the removal of a single increment, but they were generally computed subsequently to terms of milliliters per hour (ml./hr.). Rates of atmometer absorption may be termed A, while those of plant absorption are termed P. Also, A_w and P_w may refer to rates secured with measured wind, and A_c and P_c to rates secured in the tunnel but without wind.

The potometer used (fig. 2) consists essentially of a horizontally placed piece of barometer tubing calibrated throughout a length of approximately 25 cm. and provided with a scale showing 1 ml. divided into twentieths. After the instrument had been connected to a culture or atmometer, distilled water which was placed in reservoirs I and H was allowed to enter through cock J so as to fill the entire system. With cock J closed, as well as any of the D tubes not in use, and with cock G open, application of a little suction at B (fig. 1) caused water to replace all undissolved air in the potometer-bottle assembly. During periods of experimentation, an atmometer was usually attached to one D tube, a culture bottle to another, and the third was closed with a piece of rubber tubing and a clamp. After the whole assembly had been filled, cock B was closed, and any water subsequently removed from the bottle by plant or atmometer was naturally replaced by movement from reservoir I. A small air bubble, to serve as index, was introduced through tube F. After each measurement, before the bubble reached the lateral tube leading to reservoir H, cock J was temporarily opened, to permit entrance of water from H, so that the bubble was driven back beyond the zero of the scale; the instrument was thus quickly made ready for the next measurement. The potometer tube was frequently cleaned with chromic acid cleaning solution, to avoid possible distortion of the air-bubble index.

It is generally impossible to expose in a room two or more stationary objects, such as plants or atmometers, so that they are subjected to really like sets of environmental conditions (**4, 16**). WILSON (**17**) observed great differences in evaporativity in different parts of the same greenhouse room; there is usually considerable air movement at different and fluctuating rates, and in various and changing directions, due to such causes as temperature differences or differences in air pressure between windward and leeward sides of the room. Furthermore, really calm air is practically impossible to obtain. GIDDINGS (**3**), and BLACKMAN and KNIGHT (**1**), who have discussed some of the problems involved in attempts to compare transpiration and evaporation in freely moving air, have described methods for avoiding some of the difficulties thus encountered. For such compari-

sons artificial wind from an electric fan cannot be employed without a suitable wind tunnel.

The tunnel used by us was similar in many respects to the one described by BLACKMAN and KNIGHT (1). It was horizontal and stood in a greenhouse room. It was mostly made of builders' wall-board ("celotex") supported on the outside by a light wooden frame. It consisted of a frontal portion about 75 cm. square in cross section and 2 meters long. Continuing this was a tapering portion 1.5 meters long and 45 cm. square at the smaller end, with a circular central opening just large enough to allow the propeller of a suitably supported 40-cm. electric fan to rotate in it, drawing air out of the tunnel. The top or ceiling of the frontal portion consisted largely of glass, to permit entrance of light. A suitable sliding panel in the floor of the frontal portion permitted the introduction of a plant or atmometer in the desired position and allowed the floor to be closed around the stem or supply tube. The bottle was supported beneath the tunnel and the potometer was conveniently placed outside, at a convenient level for reading. Air flow was regulated by means of a hand-operated rheostat which controlled the fan. The available range of air-flow rates was from about 1 km./hr. to about 26 km./hr. Rates of air flow were measured by means of a Mason anemometer placed in the center of the frontal section of the tunnel, and a thermometer suspended near by indicated air temperature. Wind velocities were expressed in terms of miles per hour (mi./hr.). The air of the tunnel around plant or atmometer was regarded as calm when the fan was not in operation, although there was surely always significant but unmeasured air movement under these conditions, especially upward and downward convection.

When a series of measurements was to be started, the rate of air flow was first adjusted; then the anemometer was removed from the tunnel, and an atmometer assembly was quickly introduced, the tunnel floor being closed around the atmometer tube. Several potometer measurements of atmometer absorption (A_w) were then made as quickly as circumstances would permit, after which the atmometer assembly was replaced by a plant culture and a number of potometer readings of plant absorption (P_w) were obtained. The fan was then stopped, and potometer readings with calm air were secured, first for plant (P_c) and then for atmometer (A_c). It required only a few minutes to replace the atmometer by a plant, and conversely.

The potometer lag

It was found that the full effect of any pronounced alteration in air movement past plant or atmometer, which would measurably change the rate of transpiration or evaporation, was not immediately shown in terms of bubble movement; there was always some lag. After each change—

from calm to wind, from wind to calm, or from one measured wind velocity to another—it was necessary to secure several successive potometer readings throughout an adjustment period, until a steady rate of progress, corresponding to the new air conditions, had been attained. That period which is the subject of the present contribution, will be termed lag period. As might be expected, it was always shorter for the atmometer than for a plant under similar air conditions, and it varied in length according to time of day, weather, etc.

Since the liquid of the culture bottle or the atmometer sphere was continuous with the water column in the potometer tube, and since this liquid system was bound by rigid walls throughout, excepting at the menisci of the index bubble and at the open end of the tube, any movement of the bubble along the scale must have represented a corresponding change in hydrostatic pressure in the system; and that, in turn, must have represented removal of liquid from the bottle or from the cavity of the atmometer sphere, unless it were due to liquid contraction accompanying a lowering of temperature. The last clause refers to the familiar thermometer effect, which requires attention in all volumetric measurements of this sort, but temperature changes in the system could not introduce any considerable influence on bubble movement during the relatively short periods required for the timing of absorption increments of 0.1 ml. each. Volumetric rates of bubble advance along the graduated potometer tube are therefore taken as practically equivalent to concurrent rates of removal of liquid from culture bottle or atmometer sphere. These absorption rates were considered as controlled by current rates of transpiration or of evaporation, upon which air movement was effective, but the lag required attention.

For the sake of clearness it may be mentioned, without attempting a more thorough analysis here, that the rate of transpiration or evaporation at any moment is of course controlled partly by (a) internal characteristics (including temperature and exposure) of the transpiring surfaces or of the evaporating surface and partly by (b) the evaporating power of the surrounding air. Transpiration is of course subject to pronounced influences exerted by the absorbing and conducting capacity of roots and stems, as well as by structural characteristics of the leaves; but there is no internal resistance to evaporation from the atmometer sphere excepting what is introduced by the sphere wall—the atmometer tube offers no significant resistance to water movement (it has a bore of 6–7 mm.) and the instrument is without any resistance such as is due to the root system of a plant.

The water system within the pores of the atmometer sphere is apparently subject to slight readjustment whenever the rate of evaporation changes considerably, probably because of expansion or contraction of gas in small gas-filled spaces among the pores or because of slight outward or inward

movement or change in curvature of the submicroscopic menisci at the outer openings of the pores or because of both. Readjustments may also be due to volume changes in an air bubble in the sphere cavity, if one exists there. Aside from these suggested reasons for the apparent slight extensibility and elasticity of the atmometer water system, any change in evaporation rate should be accompanied by a corresponding change in the hydrostatic pressure within the sphere cavity, which should be transmitted almost instantaneously to the potometer tube, for we may be sure that no sensible change in the specific volume of water took place on account of such very slight pressure changes as might occur. The entire range of pressure fluctuation in our porous-porcelain spheres—mounted as they were without special precautions such as need to be introduced if one wishes to demonstrate negative pressures, as in the classic ASKENASY experiment—was less than one atmosphere; no truly negative pressure (*i.e.*, pressure with the *minus* sign, or traction pressure) was at any time developed, and the pressure differences here considered were consequently always within the limits of true suction, for which the liquid is at all times under positive pressure and is never "stretched." In any event, the atmometer lag was generally short.

The plant differed fundamentally from the atmometer sphere and tube in that the liquid of the former was not held within rigid walls. With decreased hydrostatic pressure produced by acceleration of transpiration, for example, the volume of the liquid system of the plant might be considerably reduced without air bubbles forming in cells or in vessels free from undissolved gas, and of course gas bubbles might form or enlarge in some vessel segments. Thus, through bending of cell walls and corresponding minute but numerous pressure adjustments—in leaves, stem, and root—the plant might give off water temporarily at a rate more rapid than the concurrent rate of liquid absorption through the roots. If the transpiration rate is maintained after such an acceleration, these adjustments should soon terminate in a new set of pressure gradients throughout the plant, and then absorption and transpiration should again become equal and might continue so indefinitely. In a similar manner a reduction in transpiration rate (as when the air condition is changed from that of a considerable wind to that of calm) should be followed by a period of adjustment in the reverse direction, during which absorption should temporarily exceed transpiration. Such adjustment periods, with which students of plant transpiration and absorption have long been familiar, are probably the lag periods considered here. The liquid system of the plant thus acts, on the whole, as though it were extensible and elastic. In our tests, considerable increase or decrease in wind velocity apparently increased or decreased transpiration or evaporation almost instantaneously, but the lapse of the

lag period was required before the potometer readings came to represent the transpiration rate or the evaporation rate that corresponded to the new air condition.

The potometer lag cannot be related to any hydrostatic-pressure readjustments outside of the plant itself or outside of the atmometer sphere. As soon as a pressure change occurs in the culture bottle, or in the sphere cavity, it should be quantatively transmitted to the potometer. Thus, although potometer readings taken within the lag period represent current rates of absorption by plant roots or by sphere, they do not represent precisely either current transpiration or current evaporation rates.

Plants of *Salix purpurea* and *Cephalanthus* were used in the representative experiments here described, the wind velocity employed being 4.4 mi./hr. unless otherwise stated. The length of the lag period for transpiration of both kinds of plant was found to be about the same, other conditions being alike. It was also about the same, other conditions being the same, whether the air change was in the direction from calm to wind or in the opposite direction, but in the latter case it sometimes appeared to be somewhat longer because the 0.1-ml. increments of absorption immediately following the stopping of the fan required much longer periods than were required when the fan was in operation. It was found that the length of the lag period was longer as the preceding steady rate of absorption for calm air had been slower, and conversely, thus showing a fairly clear diurnal march. In very early morning and late evening the period was longest, being shortest about midday, when the steady absorption rate for either calm or wind was at or near its maximum for the day. Furthermore, the amount of water absorbed during the lag period was greater as the period was shorter and as the absorption rate in calm air was more rapid, the average rate of water absorption during the lag period being most rapid about midday and least rapid in early morning and late evening. The length of the lag period was apparently not generally and consistently related to the accelerating effect of wind on transpiration.

For example, at about 6 A. M., 11 A. M., 4 P. M. and 9 P. M., on a clear day, the lengths of the mean lag periods for basket willow plants were respectively about 8, 3, 7 and 12 min., and the corresponding volumes removed from the potometer tube during the lag period were about 0.55, 0.75, 0.60 and 0.35 ml. Absorption rates in calm air were, respectively, 1.46, 6.0, 2.00 and 0.72 ml./hr., and accelerations induced by 4.4-mi. wind were 45, 30, 36 and 14 per cent.

For a second example, readings between 8 and 10 A. M. on three similar clear days, with air temperature about 20° and relative humidity about 66 per cent., gave the averages shown below.

PLANT USED	STEADY ABSORPTION RATE, CALM AIR	WIND ACCELERATION	CHANGE IN AIR MOVEMENT FROM	APPROX. LENGTH OF LAG PERIOD	APPROX. LOSS FROM POTOMETER DURING LAG PERIOD
	ml./hr.	%		*min.*	*ml.*
			Calm to wind	7.3	0.35
Salix	2.8	43	Wind to calm	6.8	0.45
			Average	7.1	0.40
			Calm to wind	6.7	0.45
Cephalanthus	2.4	88	Wind to calm	7.5	0.35
			Average	7.1	0.38
			Calm to wind	1.3	0.15
Atmometer	1.1	555	Wind to calm	5.6	0.1
			Average	3.4	0.1

As to the error that might be introduced if the potometer lag were neglected, a few numerical values may serve to show its order of magnitude; these are taken from the series of data on which was based the example just given. When a *Salix* plant was absorbing in calm air at a steady rate of about 2.8 ml./hr. the fan was started, giving a wind velocity of 4.4 mi./hr. Then the next three 0.1-ml. increments of absorption required, respectively, 1.84, 1.65 and 1.53 min.; thus the first reading after the fan was started showed a rate of 3.27 ml./hr., the second showed a rate of 3.64 ml./hr., and the rate shown by the third was 3.91 ml./hr. The steady rate for wind was about 4.00 ml./hr., and the first reading was therefore too low by about 18 per cent. For the reverse test, with the plant in wind and showing a steady absorption rate of 4.00 ml./hr., the fan was stopped, after which the next three potometer increments represented absorption rates of 3.6, 3.1, and 2.8 ml./hr., respectively, the latter being about the steady rate for calm air. The first reading after the fan had stopped was too high by about 30 per cent. These and other percentages of error that would be introduced by neglecting the lag following a change from calm to wind or from wind to calm are assembled below:

	CHANGE FROM CALM TO WIND	CHANGE FROM WIND TO CALM
Salix	18 per cent. too low	30 per cent. too high
Cephalanthus	25 per cent. too low	50 per cent. too high
Atmometer	Error too small to measure	13 per cent. too high

It is thus seen that the lag under discussion is apt to be of considerable importance, especially with regard to plant absorption. To avoid error due to it, the time periods required for the removal of successive 0.1-ml. incre-

ments from the potometer, after a change in air movement, were always recorded until a steady rate was attained, and that steady rate was then taken to represent the new air condition, as has been said. In this connection it should be noted that the hours of the day mentioned in this account represent the times when the respective tests were started. It usually required less than 20 min. to allow for the lag period in any test and to obtain the value of the steady rate; but when air humidity was high and rates of transpiration and evaporation were consequently slow, the time required for ascertaining all the several rates recorded for a given time was sometimes as long as 1 hr.

Data for an additional example of these lag relations are shown graphically in figure 3. Tests were made, using 0.05-ml. increments, at about 6 and 11 A. M. and at about 4 and 9 P. M., at which times the acceleration due

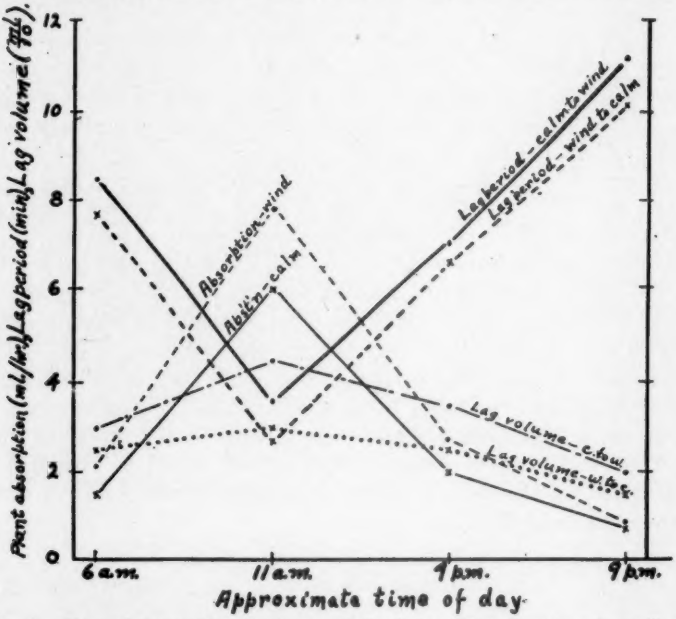

FIG. 3. Rate of absorption of culture solution by a willow plant in calm and wind at different times of day, the corresponding length of the lag period in a change from calm to wind and wind to calm, and the volume of solution absorbed during these periods.

to wind amounted to 45, 30, 36, and 14 per cent., respectively. The plant used was a basket willow and the wind velocity was 4.4 mi./hr. The steady absorption rates, for calm air and wind (the latter being of course the greater) are seen to have been maximal at about 11 A. M., which is also true

of the lag volume (amount of absorption occurring during lag period), but the length of the lag period is seen to have been minimal at that time. In this instance the lag period and the lag volume were a little greater for the change from calm air to wind than for the change from wind to calm air.

MARTIN and CLEMENTS (**10**) found that a pronounced and rapid initial acceleration of transpiration occurred when their weighed sunflower phytometers were subjected to artificial wind after a period of calm. Their first measurement of the rate of water loss was not made until 15 min. after the fan had been started, but they were convinced that the maximal transpiration rate had been attained and passed in that first period. It seems probable that their plants, with roots in moist soil, did not attain their maximal rate of water loss more quickly than did our plants, with roots in solution. The lag period for basket willow, with which most of our tests were made, was usually less than 10 minutes.

It seems clear that, as has been remarked, the potometer lag for our plants represents hydrostatic readjustments of some sort within the plant body and that the lag period represents the time required for the propagation of a hydrostatic-pressure change from transpiring membranes in the leaves to the external solution bathing the absorbing membranes at the root surfaces. These readjustments probably occurred mainly in cortical tissue, especially in the leaves, in the experiments thus far considered; they might, however, occur in cortical tissues elsewhere in the plant, as in stem and roots, and of course they might occur in the xylem vessels.

To gain some insight concerning stem-length influence on the potometer lag, a series of comparisons was made between long-stemmed and short-stemmed basket willow plants. The short-stemmed plants had stems about 30 cm. long, while the long-stemmed plants had stems about 120 cm. long. These tests on stem length were otherwise regular in every way and all plants were essentially alike with respect to foliage and root systems. A wind velocity of 6.6 mi./hr. was used in comparison with calm air. Some representative results follow:

		SHORT STEMS (30 cm.)	LONG STEMS (120 cm.)
Steady absorption rate in calm air (ml./hr.)		4.9	4.7
Acceleration due to wind (per cent.)		34	36
Length of lag period (min.)	Change from calm to wind	7.1	8.2
	Change from wind to calm	7.1	8.0

The lag period for the 120-cm. plants was thus about 14 per cent. longer than for the 30-cm. plants and it seems clear that this difference was due to difference of stem length. Supposing that stem resistance to propagation of a hydrostatic pressure change from leaves through roots was proportional to stem length, and supposing that resistance due to foliage, or to foliage and roots taken together, was alike in both cases, then the equivalent of 14 per cent. of the shorter lag period (or 0.98 min.) may be considered as having been due to 90 cm. of stem in the long-stemmed plants. Accordingly, the length of lag period due to leaves or to leaves and roots may be taken as 6.7 min. in both cases, to which is to be added, for stem influence, 0.33 min. (short stems) or 1.33 min. (long stems). This reasoning leads to the plausible supposition that only about 5 per cent. of the shorter period and only about 16 per cent. of the longer period is to be attributed to stem resistance. On this basis, the stems would apparently have had to be about 630 cm. long to make the stem lag as great as the leaf-root lag. Of course these tentative computations indicate only the general order of the magnitudes considered and the percentages shown would be expected to fluctuate with the transpiring capacity of the plant considered, which was apparently about the same for both kinds of plants in these tests, and also with the prevailing moisture and temperature conditions of the air—which were also essentially constant in the tests here considered. In general, it seems safe to suppose that the lag in our regular experiments with short-stemmed plants was mostly due to readjustments occurring in the leaves, or perhaps in leaves and roots together, and that stem readjustments—although not entirely absent in the short stems—were of little importance in determining the length of the lag period or the volume of water absorbed during that period.

Any alteration in the root tissues that would increase resistance to the entrance of water from the surroundings through these tissues into the vessels, or any change in the liquid surrounding the roots that would increase external resistance to absorption, might be expected to increase the length of the lag period here considered, and this thought led to some tests on the influence of sudden changes in some chemical and physical characteristics of the solution about the roots of our solution cultures. A number of students (**2, 11, 12, 15**) have found that acids and alkalies at suitable low concentrations increased the absorption rate but that it was decreased when higher concentrations of these or other solutes were employed. MUENSCHER (**11**) found that transpiration was greatly retarded when a dilute nutrient solution about the roots was replaced by a sufficiently more concentrated one. REED (**12**) found that some organic acids and salts, when present around the roots at suitable concentrations, accelerated transpiration, whereas suitable concentrations of some organic acids retarded it.

The introduction of solutes into the liquid around plant roots may retard the absorption rate through an increase in the osmotic value of the medium or through specific physiological or chemical effects on the absorbing cells of the roots—or even on other tissues, as of stem and leaf. In the first case the solutes would act physically, by remaining outside of the roots and hindering water entrance. In the second case they would penetrate into the root tissue before producing their effect. Several possible ways might be suggested by which a chemically active substance that has already passed into the plant across the absorbing surfaces of the roots might influence water absorption through action on living cells in roots, stem, or foliage. WILSON and RUNNELS found that spraying leaves with Bordeaux mixture and various other materials (**19, 20**) increased the transpiration rate, and that a wide variety of chemical compounds increased the rate of desiccation of plant tissue (**14**). Placing various inorganic materials on the leaves produced an effect opposite to that produced when other substances were introduced into the solution around the roots, although the principles involved were perhaps similar.

In some of our experiments, the steady absorption rate of a willow plant was first ascertained, for calm air and for a 4.4-mi. wind, while the roots were in distilled water, after which the plant was transferred to another bottle filled with 0.01-M solution of HCl or KOH, or with a stronger solution of sucrose or KNO_3, and the new absorption rate was then measured as quickly as possible, first in calm air and then with wind. Different individual plants were used for the different solutions.

Absorption was markedly retarded in every instance. The retardation was computed in each case as a percentage on the basis of the corresponding absorption rate recorded just previously for the same plant when in distilled water. With 0.01-M HCl and with 0.01-M KOH absorption was very soon retarded about 14 per cent. in calm air and about 16 per cent. in the 4.4-mi. wind. This retardation cannot be regarded as due to osmotic influence of the solutions, for the osmotic values of these were very much less than the osmotic pressures commonly prevailing in the cells of healthy roots. Retardation supervened so quickly that it could hardly have been brought about through chemical effects of the solutes upon stem and leaf tissues after passage through the root cortex into the vessels. It was probably due to some chemical influence of these substances on the cortical tissue of the roots, which resulted in increasing the resistance of that tissue to penetration of water through it into the vessels, or perhaps in decreasing the activity of that tissue in driving water into the vessels—if any such activity was present. Apparently HCl and KOH (or their ions) penetrated into the cortical cells of the roots almost instantly when the root system was placed in the acid or alkaline solution, there causing rapid and considerable

lowering of the absorbing capacity of the root surfaces. It is clear that such an increase in root resistance to water entrance would lower the hydrostatic pressure throughout vessels and foliar tissues, and retardation of transpiration would be expected to result, as through incipient drying of the leaves. We wish to emphasize merely that these very weak solutions of HCl and KOH did retard absorption promptly and considerably and that their primary effect must have been chemical rather than osmotic, probably upon roots rather than upon stems or leaves. We have no evidence concerning their effect, if any, upon transpiration, but it may be presumed that water loss was retarded when root resistance to absorption was increased and absorption rate was reduced.

With 0.4-M and 0.5-M solutions of sucrose and with 0.5-M solution of KNO_3, definite values for the absorption rate could not be obtained; absorption was immediately decreased to a marked degree, but its decrease continued for many minutes, at a progressively slower rate of retardation; *i.e.*, the lag period was indefinite. Therefore the results obtained are no more than approximations. In these cases the new rate was recorded as that of the fifth 0.1 ml. removed from the potometer after the plant had been transferred from water to solution. These more concentrated solutions were surely hypertonic to the root cells (**25**), for they were observed to produce flaccidity (plasmolysis) in the terminal regions of the ultimate root branches.

The percentage retardations found for sucrose solutions with concentrations of 0.1, 0.2, 0.4, and 0.5 were, respectively, 38, 54, 68, and 76 with calm air, and about the same with wind—namely, 41, 58, 71, and 70. The 0.5-M concentration of KNO_3, which was the only solution of this salt tested, gave retardation percentages of 62 (calm) and 75 (wind). These percentages are similar to those observed with 0.4-M solution of sucrose.

From the results reported by many writers on plasmolysis and turgor changes induced by solutions of sucrose and of KNO_3, it seems safe to suppose that the absorption retardations produced by these substances in our tests were primarily due to osmotic rather than to chemical characteristics of the solutes in question. Our thought is that the retardations observed with the weaker sucrose solutions may represent nothing more than the effects of environmental osmotic resistance to water absorption by the plant roots, somewhat as though the roots had been brought into soils of inadequate water-supplying power. In the stronger solutions root injury was probably mainly or entirely due to hypertonic environmental osmotic pressure rather than to chemical stimulation or toxic influence.

It is clear from the percentage values just set forth that, for a given set of air conditions, absorption was slower as the sucrose solution was more concentrated, but it is equally clear that retardation of absorption was not

proportional to sucrose concentration nor to the osmotic value of the sucrose solution used. As has been noted, the full effect of the higher concentrations of sucrose was not registered, which may have been due, in greater or less degree, to slow penetration of this eminently non-toxic substance into the root cells and even beyond them.

The brief report of LIVINGSTON, HEMMI, and WILSON (**8**), concerning their experiments on the hydrostatic relations of soil moisture to plant growth and to the water-supplying power of the soil, suggested that a mercury barostat might be employed to maintain low hydrostatic pressures in our culture bottles, thus substituting the hydrostatic influence of a mercury column—as in LIVINGSTON'S porous-porcelain auto-irrigator (**6**) and in WILSON'S (**18**) and RICHARD'S (**13**) irrigator pots—for the above-mentioned osmotic influence of sucrose solutions. This suggestion proved impracticable however; with a mercury column exerting a suction of 0.1 atmosphere on the nutrient solution in the culture bottle, the rate of absorption by the plant was not appreciably reduced, and when the barostat was arranged to give a suction of 0.2 atmosphere, air found its way into the bottle, *via* lenticels of that part of the plant stem which was within the bottle, and prevented potometric measurement of the absorption rate. As has been seen, our plants absorbed water regularly from sucrose and KNO_3 solutions representing osmotic pressures much greater than 0.2 atmosphere, and it seems certain that regular absorption from weak nutrient solution with greatly reduced (or even negative) hydrostatic pressure might have occurred had it been practicable to prevent air entrance into our apparatus from the plant.

These relations concerning the lag in potometer response to wind and to changes in the characteristics of solutions about the plant roots may be worthy of much more elaborate and prolonged experimental analysis.

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HYDROGEN ION CONCENTRATION AND SEXUAL EXPRESSION IN *LYCHNIS DIOICA* L.[1]

J. FISHER STANFIELD

(WITH TWO FIGURES)

Introduction

Considerable work has recently been done on the physico-chemical differences between staminate and pistillate plants of dioecious species in an attempt to formulate an objective statement of the chief sexual contrasts (**4, 16, 19, 26, 27, 28**). Although not purely physico-chemical in nature, closely related work stresses the genetical and morphological aspects of the problem (**2, 3, 8, 20, 22, 37**). Earlier physico-chemical investigations most commonly involved gross analyses either of entire plants or tops, little study having been made of separate parts or localized tissues. Hydrion studies, like other determinations of tissue fluids, have generally been limited to analysis of sap expressed from entire tops of the two sexes, a procedure which leaves much to be desired as to precision and reliability. The general usefulness of recently developed vital staining techniques and of microchemical tests on living tissues suggested that their application to dioecious plants might improve materially our present understanding of the physiology underlying sexual dimorphism, especially if such determinations were compared with analyses of expressed sap.

Methods

Initial determinations reported herein involved tests on sections of living material by the range indicator method (R.I.M.) of SMALL (**29**) and on sap expressed from entire tops, minus buds and flowers. Only leaves were available in the rosette plants since no obvious stem had developed.

The range (**5, 29**) of the indicator used (fig. 1) was determined potentiometrically in well-buffered solutions. No arbitrary ranges were set. In each case the pH range given represents the definite appearance of the color which is indicative of the pH in question. This color is taken from a very thin layer of the indicator which will account for any variations from the standard ranges which are determined with a much thicker layer of liquid. Thus, for example, orange appears to be yellow when viewed in a very thin layer. Indicators in 5 per cent. alcohol were used in concentrations of 0.01 to 0.04 per cent. Free hand sections of fresh tissues were used and checked with control sections for natural coloration. The sections were left in the indicators for 8 to 10 hours. All collections were made in the morning

[1] Contribution no. 52 from the Biological Laboratories of Knox College.

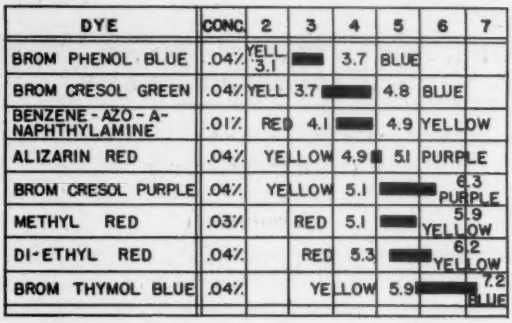
DYE
CONC.
2
3
4
5
6
7
BROM PHENOL BLUE .04% YELL 3.1 3.7 BLUE
BROM CRESOL GREEN .04% YELL 3.7 4.8 BLUE
BENZENE-AZO-A-NAPHTHYLAMINE .01% RED 4.1 4.9 YELLOW
ALIZARIN RED .04% YELLOW 4.9 5.1 PURPLE
BROM CRESOL PURPLE .04% YELLOW 5.1 6.3 PURPLE
METHYL RED .03% RED 5.1 5.9 YELLOW
DI-ETHYL RED .04% RED 5.3 6.2 YELLOW
BROM THYMOL BLUE .04% YELLOW 5.9 7.2 BLUE

FIG. 1. Ranges and colors of indicators used in determinations of pH by the range indicator method. The critical ranges are indicated by black bars.

before nine o'clock. Examinations of the tissues were made under the low power of a microscope by means of artificial light to insure uniformity. A Corning glass globe with a clear, blue Mazda lamp of 75 watts was used as a source of light. No coverslip was used over the sections owing to the possibility of acidification. A culture slide with a coverslip may be used satisfactorily however. Since this method seeks merely to determine the approximate range of pH, only definite colors were required, these colors being the end points given (fig. 1).

Further comments on this method and its results are given in an earlier paper by the writer and others (**5, 18, 24, 30, 31, 34, 35, 36**). A complete and critical description of the R.I.M. has been given by SMALL (**29**).

In making tests potentiometrically on expressed sap all materials were frozen for several hours in dry ice in a specially constructed container. The sap was extracted by means of a hydraulic cylinder press under approximately 10,000 lb. per square inch. Determinations were made immediately after extraction. In all comparative determinations made, the plants of opposite sexual expression were grown and tested under identical conditions. *Lychnis dioica* was selected for these studies because of the marked sexual contrasts of the plant, its definite dioecism, the infrequency of sex reversals, and the abundant previous work on the cytology of sex in the genus. Physiological study supplemental to existing cyto-anatomical data is required to clarify the functional changes preceding the anatomical transitions of the two sexes. Such studies should enhance our understanding of sexual differentiation and its ultimate expression in this species in pistillate and staminate parts each with its characteristic metabolism.

The data from the various tests are presented under two main headings: (1) range indicator tests, and (2) potentiometric tests.

Data

Range indicator method determinations

Comparative pH differences by the use of the range indicator method are given (table I) for entire stems of actively blooming staminate and

TABLE I

Differences in pH range of stem tissues of staminate and pistillate plants of *Lychnis dioica* L.

Tissue	Range of pH of plants	
	Pistillate	Staminate
	pH	*pH*
Epidermis	3.8–5.2	4.8–5.2
Cortex	4.8–5.2	4.8–6.0
Sclerenchyma	3.8–5.2	4.8–5.2
Pericycle	4.8–5.2	4.8–6.0
Phloem	4.8–6.0	5.0–6.0
Xylem	3.8–5.2	4.8–5.2
Pith	4.8–5.2	5.0–6.0

pistillate plants. Tests were made on both sexes of 20 different plants grown in the greenhouse. The figures given represent the definite tendency in terms of the R.I.M. In general, the staminate tissues show a higher pH than the pistillate in practically every case. The epidermis, sclerenchyma, and xylem are always more acid than the other tissues in the stems of both sexes. The test on the phloem represents a regional reaction rather than that of a specific tissue.

Comparative data for staminate and pistillate flowers are given (table II). Photomicrographs of the flowers indicate the relationship of floral parts used for the determinations and comparisons (fig. 2).

TABLE II

Differences in pH range of flowers of staminate and pistillate plants of *Lychnis dioica* L.

Pistillate flowers		Staminate flowers	
Floral part	Range of pH	Floral part	Range of pH
	pH		*pH*
Placenta	4.8–5.2	Vascular strands	5.0–6.0
Vascular strands	5.0–6.0	Pseudo-receptacle	5.0–6.0
Ovule	5.0–6.0	Base of filament	4.8–5.2
Ovary wall	4.8–5.2	Base of petals	4.8–5.2
		Rudimentary pistil	5.0–6.0

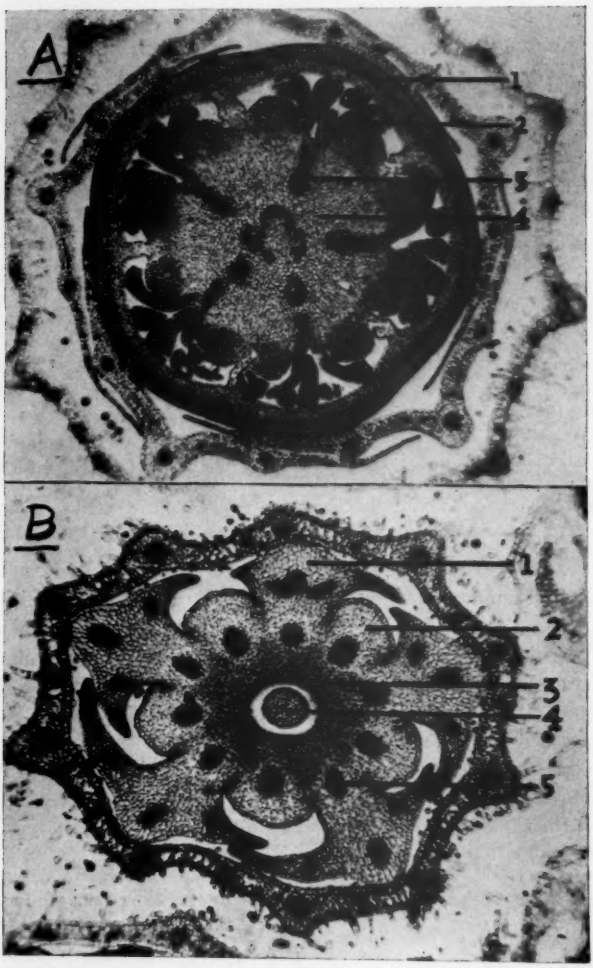

FIG. 2. Cross-sections of *Lychnis dioica* flowers. A. Cross-section of pistillate flower: 1, ovary wall; 2, ovule; 3, vascular strands; 4, placenta. B. Cross-section of staminate flower: 1, base of petal; 2, base of filament; 3, pseudo-receptacle; 4, rudimentary pistil; 5, vascular strands.

It will be noted that in the staminate flower the receptacle and its associated parts are more alkaline than either the stamen bases or the corolla lobes. The pH of the rudimentary pistil found in the male flowers (7) is the same as that of the pseudo-receptacle although differentiated from

it morphologically. Owing to difficulties in preparing material, no tests were made on the calyx. The most striking condition noted in the tests on the pistillate flower is the alkalinity of the ovules and the vascular strands as contrasted with the ovary wall and the placenta. In all tests made, this relationship remained definitely established although subject to minor variations. It was found that certain parts of the flowers of opposite sexes have the same pH range. For example, the receptacle and vascular strands of the staminate flower show the same pH range as do the ovules and the vascular strands of the pistillate flower. In like manner, the ovary wall of the pistillate flower and the base of the petals and stamen filaments of the staminate flower have identical pH ranges. The pseudo-receptacle of the staminate flower is consistently higher in its pH range than the placenta of the pistillate flower. This is of some significance since these structures occupy the major portion of the central area of each type of flower and because their mass dominates the appearance of the entire cross-section. Contrary to the condition found in the stem tissues, studies of the floral parts, as indicated above, do not show any consistent tendency toward a higher pH for either sex. Thus all differences must be expressed only in terms of individual floral parts, not in terms of the flower as an organ. Such differences in the various portions of the flower, however, are extremely consistent and therefore of diagnostic value equal to any obtained through the study of stem tissues.

Potentiometric determinations

In these tests, all parts of the plant above-ground were used with the exception of flowers and buds (table III). *Lychnis* is a long-day plant, and

TABLE III

Difference in pH of expressed sap of pistillate and staminate plants of *Lychnis dioica* L.

Materials tested	pH	
	Pistillate	Staminate
Rosette, pre-blooming stage, in greenhouse	6.8	6.8
Young plants, early blooming stage, in greenhouse	6.4	6.0
Actively blooming stage, in greenhouse	6.6	5.5
Late blooming stage, in greenhouse	6.3	5.0
Post-blooming stage, in greenhouse	6.7	6.8
Late blooming stage, in garden	5.9	5.1

it is thus possible to keep it in a vegetative, or rosette, stage indefinitely by growing it under short-day conditions where the day length is less than

13–14 hours. Such plants can be grown in all seasons under the usual greenhouse conditions of light and temperature. The plants listed as rosette plants had thus shown no sexual differentiation and were forced into bloom and the sex determined after these tests were made. This would indicate what their sex would have been if they possessed certain inherent qualities in the rosette stage which would predetermine their sexual expression when forced into bloom. Before sexual differentiation, there is no difference in the pH, and the same is true for the post-blooming stage. It is only in the blooming stage that a difference in the pH occurs. The pistillate plants are always more alkaline than the staminate in all stages of flowering and under all growth conditions. The floral parts were not included in making these tests.

There was a pronounced drop in the pH of the staminate plant upon blooming. The tests, as a whole, run somewhat higher in their pH than do the tests on similar plants with the R.I.M. An increase in acidity concomitant with the age of blooming plants is noted, particularly in the staminate plants. Young plants 10 to 14 weeks of age which have just come into bloom, as a rule, show a higher pH than do those which have been blooming for a longer period. During the blooming period, all such plants, regardless of sex, are more acid than any plants during either their pre- or post-blooming existence. Again this is more striking in the case of the males.

Discussion

Few available data deal with the physiological properties of *Lychnis*. KEEGAN (**17**) worked with this plant, but none of his analyses apply in any way to the dioecious condition found in this form. No comprehensive study of the physiological aspects of sex differentiation in this plant have been found in the literature, and hence comparison is possible only with the results obtained from other plants. SATINA and BLAKESLEE (**25**), in their work on the biochemical differences between sexes in green plants, found that the female plants of *Rhamnus, Morus, Ailanthus,* and *Rumex* were higher than the males in total acidity as determined roughly by titration with potassium hydroxide. pH determinations on the sap of *Rhamnus, Morus,* and *Cannabis* showed no significant sexual differences. HOXMEIER (**13**), in an unpublished work on the buffer capacity in relation to sex in dioecious plants, found that the expressed sap of mature male plants of hemp (*Cannabis sativa*) was more acid than that of the female and had a better buffer capacity. The buffering was more marked against acid than base. TALLEY (**32**), in a study made on the same plant, found that the males were more acid and better buffered than were the females, although the females fluctuated less in their pH. He worked only with fully matured plants. In a later work (**33**) he also found the active acidity of expressed

sap of staminate plants to be greater than that of female plants. LOEHWING (19) found that the tops of 15-week-old spinach plants were slightly more acid in the case of the female plants. Likewise in hemp he found that the tops of 10-week-old plants showed a slightly higher pH for the males. This difference was still more marked in plants 7 weeks of age. Such potentiometric studies show that the different sexes of some dioecious plants vary in their respective concentrations of hydrogen ions. The pH as such is no criterion of sex in general but in comparative tests on opposite sexes of the same species differences are shown.

ATKINS (1), using micro-colorimetric methods for the determination of pH values, found that the xylem was more acid than the pith and medullary rays, and that the midrib of the leaf was more acid than the parenchyma. ROGERS and SHIVE (24), using the R.I.M., noted that the pith, phloem, and cortex were more alkaline than the other tissues in the stem of *Oxalis;* the phloem, in particular, was more alkaline in the stems of *Rumex* and *Zea.* A review of the literature would indicate that SMALL and his collaborators (29), in their use of the R.I.M., have the most extensive and detailed summary of the results on the variation of the pH of plant tissues. These results all indicate differences not only in the pH of extracted sap of plant organs but also in the tissues of these organs by means of the R.I.M. It is thus to be expected that similar variations might be found in the determinations given herein, and such is the case.

The results of the present study show (table III) that in actively blooming plants of *Lychnis,* the expressed sap of the staminate plants is more acid than the sap of the pistillate plants. A similar condition with regard to the expressed sap of *Cannabis* was found to exist by HOXMEIER (13) and TALLEY (33). Both of these workers used the entire plant above-ground. LOEHWING (19), however, found that, in the case of spinach, the pistillate plants were slightly more acid than the staminate, and in hemp he found the same. Such variations in the findings might be due to factors in the environment under which the plants were grown, since methods of testing were the same. Similar methods were followed by the writer. It might also be due to the parts of the plants used for testing, and their age. These are both variable factors (6, 9, 10, 11, 14, 15, 21). More data are necessary to evaluate these studies. SATINA and BLAKESLEE (26) found no significant sexual differences in the pH of the sap of some plants studied (*Rhamnus, Morus,* and *Cannabis*). The above records show varying results as regards the consistency of the correlation of the pH of extracted plant saps and of their sexual expression in the various genera tested. It is to be noted, however, that in *Lychnis,* during the blooming period, the staminate plants, consistently and under all conditions of growth, have a lower pH than the pistillate plants and thus are more acid. In every case, plants compared

were grown and tested under identical conditions. On the other hand, *Lychnis* shows no difference in the pH of the extracted sap from rosette plants which have not as yet showed any sexual differentiation. The same lack of contrast holds true for the plants during the post-blooming stage. The increase in acidity of plants concomitant with their blooming is quite obvious when compared with the pre- and post-blooming stages. The actual increase in acidity in the staminate plants is much greater than that found in the pistillate plants. The male plant characteristically remains below neutral in its pH and is thus always on the acid side, according to both the potentiometric method and R.I.M.

No previous work has come to the writer's attention which treats of the R.I.M. for determining the pH of cells and tissues in *Lychnis dioica* L. except the work of SMALL (**29**) and REA and SMALL (**23**). Their determinations did not deal with possible correlations of hydrion concentration with sex in dioecious forms but were confined to tissues within the stem and flower respectively. In using the R.I.M. it is found that, in general, the stem tissues of the pistillate plants are more strongly acid than those of the staminate. In both sexes, however, it is clearly demonstrated that the epidermis, xylem, and sclerenchyma are more acid than the other tissues of the stem. This condition seems to be rather general in the stems of plants, according to ATKINS (**1**), SMALL (**29**), and ROGERS and SHIVE (**24**). It may result from a more acid condition of their cell walls due to lignification. The fact that the phloem and pith showed the least acid tendencies correlates well with the observations of SMALL (**29**) on the Caryophyllaceae, to which *Lychnis* belongs. A similar condition was found in *Oxalis, Rumex*, and *Zea* by ROGERS and SHIVE (**24**).

There are many factors to be considered in any comparison of the potentiometric method and the R.I.M. of hydrion determination. This is shown by the fact that the stems of the staminate plants have, in general, a pH higher than that of the stem of the pistillate plants with the R.I.M. Opposite results are obtained with expressed sap. That is, the major tissues of the stem of the two sexes show differences by the use of the R.I.M. that are not obvious in potentiometric determinations on the expressed sap of such a highly differentiated organ. This should not invalidate either of the results, because the ranges obtained with the R.I.M. are quite large and very nearly include the limits of the variation shown between the two sexes by the potentiometric determinations. It is probable that a refinement of the R.I.M. with smaller and more definite categories would give results more directly comparable to those obtained by the use of the potentiometric method, but a detailed discussion of the comparative worth of the two methods of testing is beyond the scope of this work. Small differences in

the pH are often difficult to distinguish by the R.I.M. due to the inability of the observer to differentiate color values in certain ranges.

Certain definite and consistent differences between the pH of tissues can however be demonstrated within the organs of plants by means of the R.I.M. The strikingly acid condition of the woody and cutinized tissues of the stem cannot be shown in expressed sap from crushed whole stem. These tissues would yield little sap in comparison with the more succulent parenchymatous tissues which normally show a higher comparative pH by the R.I.M.

Comparative studies of the floral sections by the R.I.M. indicate a more alkaline tendency in the staminate tissues. This alkalinity is conducive to an increased oxidase activity since this enzyme complex is known to function more effectively at or above the neutrality point. Determinations made by the writer on sap expressed from the floral buds of both sexes are in agreement with this fact since the staminate buds show a much greater oxidizing power than do the pistillate. The higher pH of the expressed sap of entire plants, minus buds and flowers, concomitant with femaleness in all stages of blooming, is also in agreement with this general tendency. In each of the three stages of blooming studied (table III) the pistillate plants were found to have a higher pH and to be higher in oxidase activity. (Unpublished data.) It has been further noted that the floral buds differ in a striking fashion from the vegetative parts in their oxidizing powers. The staminate plant has the higher rate of the two in the floral buds, while the pistillate plant has the higher rate in the vegetative parts. The high degree of correlation found in these tests lends further support to the correctness of the general relationship between hydrion concentration in tissues and their oxidase activity. The greater acidity of the pistillate flower, as shown by the R.I.M., appears to be due to the greater amount of oxalic acid in its major tissues, since the placenta and wall of the ovary with a low pH contain more calcium oxalate crystals than analogous parts of the staminate flower.

Summary

Range indicator data

1. The acidity of various tissues of the staminate and pistillate plants of *Lychnis dioica* was determined by means of the range indicator method, using a series of suitable indicators. Sections of the living stems and flowers were microscopically compared.

2. In both sexes, the xylem, epidermis, and sclerenchyma were consistently more acid than the other tissues of the stem. The phloem and pith were consistently more alkaline than the other tissues.

3. In general, the stems of the staminate plants show a higher pH than those of the pistillate, viz., a more alkaline tendency in all tissues.

4. The ovules and vascular strands connected with them are consistently more alkaline than other tissues of the ovary.

5. The ovary wall in the pistillate flower, the base of the stamen filament, and the base of the petals in the staminate flowers show a consistent similarity in the pH range.

6. The pseudo-receptacle of the staminate flower is always more acid than the placenta of the pistillate flower.

7. No general pH range can be said to differentiate staminate from pistillate flowers except by reference to individual tissues mentioned above.

Potentiometric data

1. Comparative tests were made on the expressed sap of *Lychnis dioica* L. by means of the potentiometer. The entire plant above-ground was used, with the exception of flowers and buds.

2. In the actively blooming stage, the expressed sap of the staminate plants is consistently and under all conditions of growth more acid than that of the pistillate plants. No recognizable differences in the pH are noted in either the pre- or the post-blooming stages.

3. A general increase in acidity is noted, concomitant with blooming, in both sexes. The staminate plants become more acid than do the pistillate.

4. No correlation between any definite pH range and the expression of maleness or femaleness is to be noted in these potentiometric determinations.

5. In general, there is an increase in acidity with age in plants of both sexes during the blooming season. The younger plants are less acid.

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NITROGEN IN RELATION TO THE GROWTH OF CITRUS CUTTINGS IN SOLUTION CULTURES

A. R. C. Haas

(WITH FIVE FIGURES)

The commercial culture of citrus in most soils of California requires some form of nitrogen fertilization. Under certain conditions nitrogen may be readily carried to depths below the root zone. In addition, with trees of large size it is frequently difficult to maintain appreciable concentrations of nitrate in the soil solution on account of the large nitrogen requirement of the trees (5).

A healthy state of growth in citrus trees frequently is associated with an extremely low concentration of nitrate nitrogen in the soil, and a poor state of growth with a relatively high concentration. In the former case the nitrate is rapidly absorbed while in the latter it is absorbed very slowly because of high concentrations of chlorides or because of some other unfavorable condition.

It is desirable, on account of the cost, to keep the nitrate level as low as is consistent with a healthy growth response. In the effort to accomplish this it is possible that the roots during certain periods may be bathed by a soil solution containing little, if any, nitrate or other forms of nitrogen. It has been shown that the growth of citrus in solution cultures is benefited by the temporary depletion of certain elements (3). The length of such depletion period, however, and the nature of the other ions in the solution, determine in a large measure whether or not actual injury shall follow.

Deficiency of nitrogen in solution cultures

Rooted leafy-twig cuttings of rough lemon were grown in a culture solution of the following composition (p.p.m.):

Na	K	Ca	Mg	Cl	NO_3	SO_4	PO_4	B	Mn	Fe
7	185	159	54	10	718	216	105	0.5	6.2	0.1

Five-gallon capacity shallow enamelware pans were used for the cultures and the solution was not otherwise aerated. It was found by testing the culture solution that nitrogen was the first element to become depleted. The culture solution[1] was renewed at frequent intervals and the cuttings were grown until they were about four feet high.

[1] In all cultures reported in this paper, phosphate was added for a short time prior to the renewal of the culture solution. When phosphate was absent, 5 p.p.m. of aluminum was added in the form of the citrate to the culture solution.

When this growth was attained, the culture solution was not renewed. After a time, the nitrate became fully depleted while a considerable amount of calcium was still present in the culture solution. This condition was soon followed by a marked response in the appearance of the cuttings.

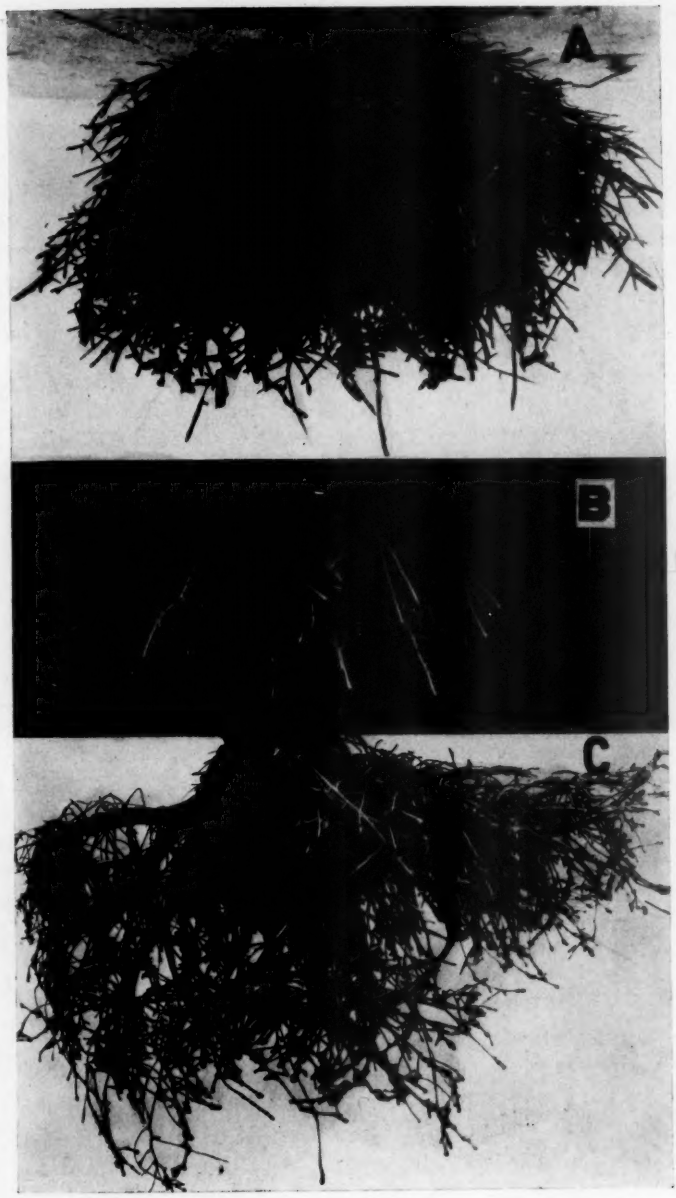
The roots became gelatinous and bluish in color. The tops showed no immediate effect, although there was a gradual loss of leaves during the period of equilibrium restoration between the top and root. The odor of decomposing roots was strong for some distance away whenever a culture vessel cover was momentarily lifted. At this stage, a solution of calcium nitrate was added to the unchanged culture solution sufficient to increase the calcium content by 159 and the nitrate nitrogen by 493 p.p.m.

Overnight the odor of the solution disappeared, the colloidal matter in the culture solution settled, and within a few days new growth was evident in the older portion of the root system as seen in figure 1. The gelatinous disintegrating portion gradually separated from the living portion of the root system and settled.

Figure 1 A shows the darkening and collapse of the roots after the nitrate nitrogen in the solution was depleted. The effectiveness of adding calcium nitrate to the unchanged culture solution may be seen in the initial growth of white root tips from the live older portion of the root system. Figure 1 B shows a stage in the later growth of the new roots and illustrates the sloughing away of the gelatinized portions. Control cultures developed excellent roots that did not undergo these changes. In these control cultures the renewal of the solutions was not interrupted except in a few cultures in which the occurrence of a period of nitrate deficiency was avoided by the addition of calcium nitrate to the unchanged culture solution. It should be stated, although the causes are not understood, that the growth of citrus cuttings is considerably better in solutions that are frequently changed than in solutions less frequently renewed but in which attempts are made to maintain given concentrations of certain elements. Causes for such a difference may be found to be associated with the concentrations of the so-called minor elements or of toxins in the solution.

The effect of a nitrate-nitrogen deficiency on the roots of leafy-twig cuttings of Lisbon lemon grown in culture solution is seen in figure 1 C. The cuttings were grown for a year without a period of nitrogen deficiency

FIG. 1. Nitrogen-deficiency and recovery effects in the roots of citrus cuttings grown in solution cultures. A, gelatinization of rough lemon roots in the absence of nitrogen and the initial stage of new root growth following the addition of calcium nitrate to the unchanged nitrogen-depleted culture solution. B, a later stage in the growth of the new roots which is accompanied by the sloughing away of gelatinized roots. C, similar effects on roots of cuttings of Lisbon lemon in which the period of nitrogen deficiency was not prolonged.


A
B
C

because of the frequent renewal of the culture solution. Nitrate was then allowed to become deficient in the manner previously described. The period of nitrogen deficiency was not unduly prolonged before calcium nitrate was added to the unchanged culture solution. New root growth from the living or least injured portion of the root system (fig. 1 C) indicated the beginning of the recovery process.

Cuttings of Valencia orange as scion, on cuttings of rough lemon as stock, were grown for a year in a culture solution containing nitrate nitrogen, and then were grown in a culture solution containing no nitrogen. The composition of the nitrate-free culture solution was as follows (p.p.m.):

Na	K	Ca	Mg	Cl	NO_3	SO_4	PO_4	B	Mn	Fe
7	185	159	54	280	0	216	105	0.5	6.2	0.1

The deficiency was allowed to continue until the growth of the roots was definitely at a standstill before calcium nitrate was added to the solution. The addition of nitrate prevented the collapse of the roots. The deficiency of nitrogen, though not for a period of sufficient duration to destroy the root system, nevertheless severely injured it. The youngest roots failed to respond to the addition of the nitrate. The growth response in the root system during the following year was, therefore, entirely from the older, less injured roots.

It was found that small, freshly-rooted cuttings of rough lemon were able to withstand a nitrogen deficiency for a long time when placed directly into the nitrate-free culture solution. The new top growth, if any, was uniformly pale green in color. When cuttings were grown to a considerable size in culture solutions containing nitrate nitrogen and were then grown for a sufficient time in a nitrogen-deficient solution, the roots were injured and the leaves reflected the root condition. The leaves in such cases became pale green, and the veins light colored (fig. 2 A) which usually signifies that the root system is injured. This light-colored vein condition is readily distinguishable from the effects known as chlorosis (fig. 2 B) in citrus leaves in which the veins are green while the remainder of the blade assumes a yellowish green color. Chlorosis may be brought about in many ways, chief among which is the factor of pH.

Nitrogen effects on the growth of cuttings

Previous investigations (Haas, 2) have shown that certain forms of nitrogen, when continuously supplied to sand or soil cultures in amounts that insure the absorption of the nitrogen in an unchanged condition, are injurious to citrus, and produce leaf symptoms as evidence of such injury. Urea, cyanamid, dicyanamid, and closely related forms of nitrogen behave in this way. In view of the fact that little, if any, knowledge exists in

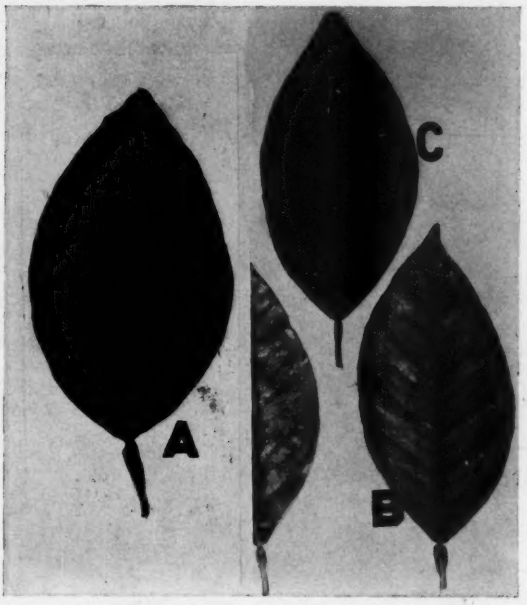


FIG. 2. Nitrogen-deficiency effects compared with those of chlorosis in citrus leaves. A, leaf from a cutting of Valencia orange as scion on a cutting of rough lemon as stock. The cutting was grown for a year in a culture solution containing nitrate nitrogen and then in a similar solution lacking nitrogen. B, leaf from a cutting of Valencia orange affected with chlorosis as a result of a high pH value in a culture solution containing nitrate. Note the reverse condition of the veins in A and B. C, leaf from a cutting of Valencia orange grown in a control culture solution.

regard to the effect of the form and concentration of nitrogen on the growth of citrus under controlled conditions, preliminary experiments were made in solution cultures as a basis for future investigations.

AMMONIA NITROGEN

Rooted leafy-twig cuttings of Lisbon lemon were grown from September 19, 1934, to April 25, 1935, in 5-gallon capacity shallow enamelware pans. The culture solution used had the following composition (p.p.m.):

Na	K	Ca	Mg	Cl	SO_4	PO_4	B	Mn	Fe
3.5	71	159	27	275	156	105	0.5	6.2	0.1

The alternation of phosphate with aluminum citrate was carried on as previously mentioned. Ammonium sulphate solution was added to the culture solution in order to secure the following concentrations of nitrogen:

0, 1, 2, 3, 5, and 10 p.p.m. The culture solutions were renewed every few days and the pH was maintained at 6 to 6.5 by means of a solution of calcium hydroxide.

Growth of the tops was increasingly better as the nitrogen content of the solution increased (fig. 3). The root system was somewhat larger at

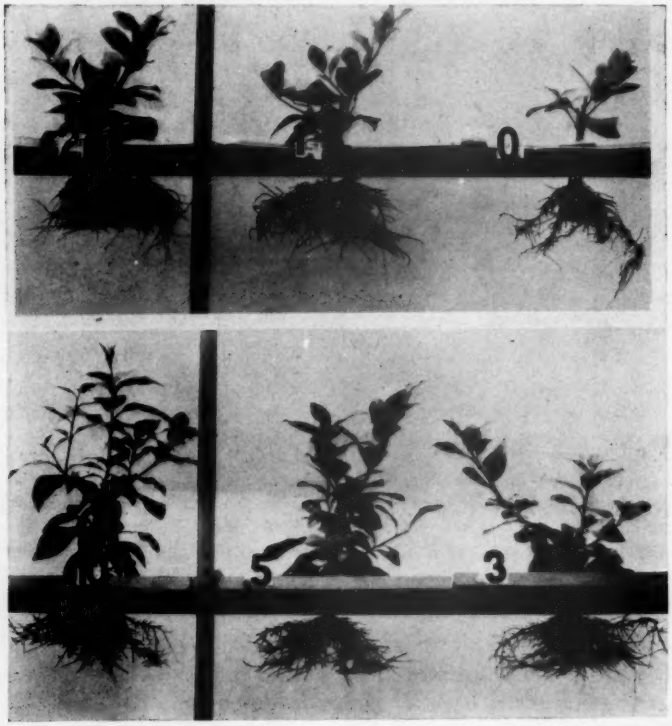


FIG. 3. Growth of leafy-twig cuttings of Lisbon lemon in solution cultures to which various concentrations (p.p.m.) of nitrogen were added in the form of ammonium sulphate.

the lower than at the higher nitrogen concentrations. In the low nitrogen cultures, some of the leaves showed slight evidence of sulphate excess (HAAS and THOMAS, 4). This is difficult to avoid because of the low nitrogen-sulphate ratio. Furthermore the absorption of ammonia in the absence of nitrate may not be entirely without injurious effects. In fact, the citrus leaves were yellowish-green in color regardless of the iron supply. The cuttings had the appearance of being in need of nitrogen. These effects of

ammonium absorption agree with those obtained in soil cultures that were given daily applications of dilute ammonia. In soil, the discontinuance of the ammonia treatment was followed by recovery as the nitrification of the ammonia progressed.

In the present solution cultures, nitrate was not found in the solution at any time, and unless the ammonia was changed to the nitrate form as it was absorbed, the only conclusion possible is that these cultures were grown with enforced ammonia absorption. It is possible, therefore, that a given concentration of ammonium becomes less injurious as the solution or the plant contains increasing amounts of nitrate. Further investigation will be necessary to secure data on the ammonia-nitrate relationship.

Nitrate nitrogen

Other cuttings of Lisbon lemon were grown in a culture solution similar to that used for ammonia nitrogen. Nitrogen in the form of potassium nitrate was added to the culture solution in order to obtain the following concentrations (p.p.m.): 0, 1, 2, 3, 5, 10, and 15. Figure 4 shows the resulting growth: that at 3 or 5 p.p.m. was greater than that at 1 and 2 p.p.m. and less than that at higher concentrations. It is doubtful, therefore, whether rapidly growing cuttings, such as lemon, can secure adequate supplies of nitrogen from solutions containing extremely low concentrations of this constituent. In the field, such low concentrations of nitrogen frequently are associated with a healthy vigorous growth in citrus but at some time prior during the year the nitrogen level in the soil far exceeded the low nitrogen concentrations.

Nitrite nitrogen

Nitrites have been reported (1) as occurring in considerable concentration in certain soils. The chemical determination of nitrites in soils and plants is a difficult one because of possible changes in the form of the nitrogen that may occur under certain conditions. There has long been a suspicion that nitrite in the field at times may reach concentrations injurious to citrus, but proof of this contention is lacking. Experiments in which solution cultures were used, furnish data regarding the effect of nitrites on citrus.

Rooted leafy-twig cuttings of Lisbon lemon were grown from July 23, 1934, to March 22, 1935, in solution cultures in 5-gallon capacity shallow enamelware pans. The culture solution contained (p.p.m.):

Na	K	Ca	Mg	Cl	NO_3	SO_4	PO_4	B	Mn	Fe
7	185	159	54	10	785	216	105	0.5	6.2	0.1

The aluminum-phosphate relation was maintained as previously. A solution of potassium nitrite was added to the culture solutions in order to secure

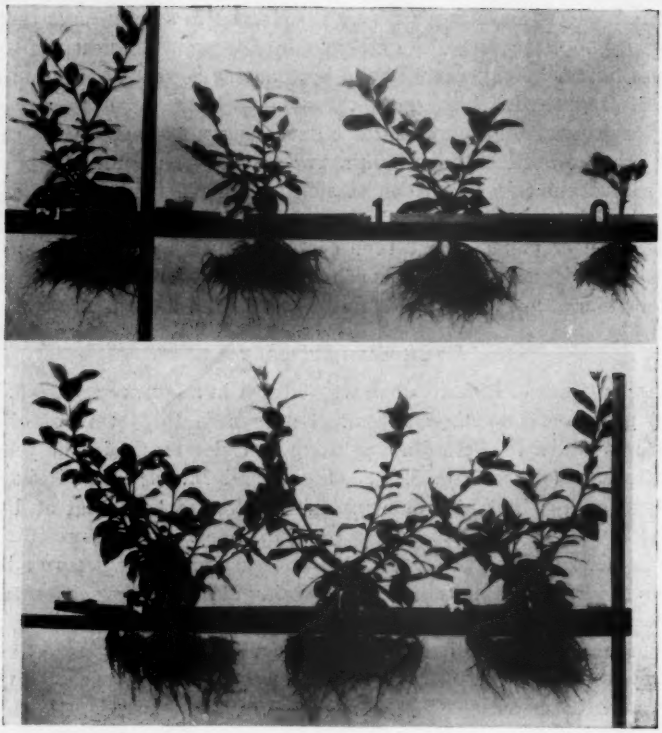


FIG. 4. Growth of leafy-twig cuttings of Lisbon lemon in solution cultures containing various concentrations (p.p.m.) of nitrogen added in the form of potassium nitrate.

the following concentrations of nitrite (p.p.m.): 0, 5, 10, 15, and 25. The solutions were renewed every few days. The nitrite concentrations were maintained by testing the solutions and adding nitrite as required. The rapidity of change of nitrite to nitrate was retarded by maintaining the pH of the culture solutions in the range 7 to 7.5 by means of a solution of calcium hydroxide. Even so, the nitrite content of the solutions changed considerably within the course of a few days.

Figure 5 A shows the growth made by the cuttings in solutions containing various concentrations of nitrite. The control cultures were not distinguishable from the cultures that received 5 p.p.m. nitrite and hence are not shown in the figure. It is significant that the presence of 785 p.p.m. of nitrate has not prevented the nitrite from seriously interfering with the growth of lemon cuttings. It is not known whether the injury would have been greater in the presence of a smaller concentration of nitrate.

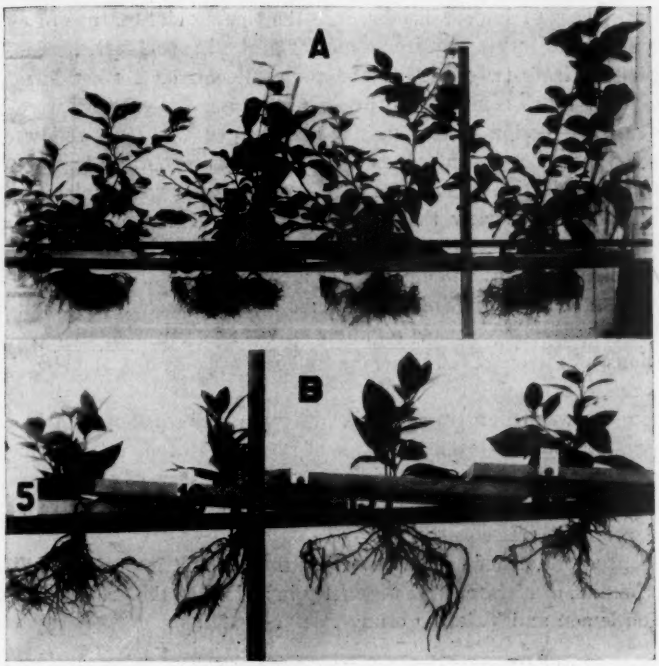


FIG. 5. Effect of various concentrations (p.p.m.) of nitrite on the growth of cuttings of citrus in solution cultures. A, Lisbon lemon; B, Valencia orange.

In a manner similar to the procedure just described for Lisbon lemon, rooted leafy-twig cuttings of Valencia orange were grown from October 26, 1934, to April 25, 1935, in solutions containing nitrite. As shown in figure 5 B the growth of the roots was interfered with when the concentration of nitrite exceeded 5 p.p.m. The cuttings in the control cultures were not different in appearance from those grown with 5 p.p.m. nitrite. Above 5 p.p.m. nitrite, the finely divided roots were usually dark in color and finally became somewhat gelatinous. Recovery was rapid when nitrite additions were withheld. The injury brought about by the presence of nitrite is not characterized by symptoms that are more or less specific, hence the diagnosis and the appraisal in the field of the injury for which nitrite is responsible are at present obscure.

Summary

1. A preliminary study was made of the growth of citrus cuttings in solution cultures with various forms and concentrations of nitrogen. Such

a study becomes of importance because the commercial culture of citrus in most soils of California requires some form of nitrogen fertilization.

2. Rooted leafy-twig cuttings of rough lemon or Lisbon lemon were grown in a so-called complete culture solution until the cuttings were several feet high. Depletion of the supply of nitrogen was then followed by a collapse of the root system accompanied by a corresponding loss of affected leaves. The addition of calcium nitrate to the nitrogen-depleted cultures brought about a recovery that varied with the degree of injury. A distinction is shown to occur between the appearance of citrus leaves in need of nitrogen and that due to chlorosis as a consequence of pH.

3. The tops of cuttings of Lisbon lemon grown in solution cultures with ammonium as the only source of nitrogen were larger with increasing concentrations of nitrogen. The leaves had the appearance of being in need of nitrogen.

4. The growth of cuttings of Lisbon lemon was greater with increasing concentrations of nitrate as the source of nitrogen in the culture solution.

5. It is doubtful whether rapidly growing cuttings of citrus can secure adequate supplies of nitrogen from solutions containing extremely low concentrations of this constituent.

6. Concentrations of nitrite above 5 p.p.m. in culture solutions that contain 785 p.p.m. of nitrate, were distinctly injurious to the growth of cuttings of Lisbon lemon and Valencia orange.

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EFFECTS OF PHOSPHORUS AND LIME IN REDUCING ALUMINUM TOXICITY OF ACID SOILS[1]

KENNETH E. WRIGHT

Introduction

There is a wide divergence of opinion as to the cause of the toxic effects of acid soils upon the growth of certain species of plants. By some this toxicity is attributed to acidity *per se*. The majority of investigators believe this toxicity is due primarily to the presence of soluble aluminum salts in the soil. Various investigators have shown that this toxic effect of acid soils can be overcome by the application of lime and of superphosphate in rather large amounts. The beneficial effects of these treatments have been variously attributed: (1) to supplying essential elements that are deficient in the plant; (2) to changing the soil reaction to a pH range that is favorable for plant growth; (3) to rendering aluminum inactive because of precipitation in the soil; and (4) to eliminating the toxic action of aluminum by supplying phosphorus in sufficient amounts both to precipitate aluminum within the plant tissues and, in addition, provide sufficient quantities for normal plant metabolism. This work was undertaken in an attempt to clarify the conflicting viewpoints just listed.

Soil culture experiments with beets

It was desirable to find some calcium salt that could be added to an acid soil without appreciably changing its pH and yet supply calcium to the plant. Thus the rôle of calcium as an essential element could be compared with its rôle in reducing acidity. Samples of an acid soil were variously treated with different amounts of calcium lactate and calcium acetate alone and in combination with different amounts of calcium, magnesium, and sodium carbonates. As a result of this experiment it was decided that the application of calcium lactate would be the most suitable for use in the reduction of toxicity as it did not appreciably alter the pH of the acid soil and yet its application resulted in plants that showed a better development than those grown in the untreated acid soil. In addition to the calcium lactate treatment acid soil was treated with lime and with superphosphate. Beets grown in untreated acid soil were used as a check.

Determinations of calcium, magnesium, phosphorus, and total nitrogen in the plant and nitrates in the soil were made according to the standard methods as described by the ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS (1). Plant nitrates were determined according to the method of FREAR

[1] Published as contribution no. 494 of the Rhode Island Agricultural Experiment Station, Kingston, R. I., by permission of the Director of Research.

(3) and total sugars and reducing sugars by the method described by SCHLENKER (7). The hydrogen ion concentration was determined by the electrometric method using a quinhydrone electrode with a saturated calomel half-cell. The results of the various analyses are shown in table I.

It is evident that pH as such is not a limiting factor in growth as the superphosphate treatment resulted in a vigorous growth of plants in contrast to the very poor development of plants grown on the acid soil, although the difference in soil pH of these two treatments was negligible. Furthermore, the calcium lactate treatment resulted in plants which showed a better general development than those of the acid soil without an appreciable change in pH.

A high calcium content of the plants grown in the acid soil indicates that a deficiency of calcium as an essential element cannot be considered as a limiting factor in growth. This is further supported by the fact that those plants grown with the calcium lactate treatment contained more calcium than the plants grown with the lime treatment, the former, however, being comparatively retarded in development.

TABLE I

ANALYTICAL RESULTS OF SOIL CULTURE EXPERIMENTS WITH BEETS

SOIL TREATMENTS	CHECK	LIME	CALCIUM LACTATE	SUPERPHOSPHATE
Ca in dry matter (%)	1.42	1.41	1.59	2.08
Mg in dry matter (%)	0.93	0.92	1.28	1.40
P in dry matter (%)	0.24	0.61	0.50	0.68
NO_3 in dry matter (p. p. m.)	264	1080	352	1140
Total N in dry matter (%)	2.98	3.85	2.87	3.35
Reducing sugars in dry matter (%)	0.86	0.60	0.38	0.79
Total sugars in dry matter (%)	1.54	1.09	1.20	1.22
Reducing sugars—juice (%)	1.35	0.33	0.30	0.21
Total sugars—juice (%)	1.48	0.36	0.34	0.29
NO_3 in soil (p. p. m.)				
7th week	48	40	22	56
8th "	60	28	14	60
pH of soil	4.32	6.38	4.47	4.49

The analyses indicate that photosynthesis apparently was not retarded because of a lack of magnesium, as the magnesium content of the plants grown in acid soil compares favorably with that of the limed flat. Moreover, there is an accumulation of both total and reducing sugars in the poorly developed plants grown in the acid soil, indicating that the carbohydrates manufactured are not being utilized by the plant. A deficiency of total nitrogen, of nitrates, and of phosphorus in the plants from the acid soil reveals possible causes of retarded growth on acid soil.

Drip culture experiments with beets

In order to determine the effects of nitrate deficiency, phosphorus deficiency, and the presence of aluminum upon the growth of beets three series of drip culture experiments were conducted. The three series of standard culture solutions used consisted of one series in which nitrates varied from 0 to 108 p. p. m.; of a second series in which phosphorus varied from 0 to 10 p. p. m.; and of a third series in which aluminum varied from 0 to 12 p. p. m. Nitrate-deficient plants before death turned yellowish and then brown. The retarded plants in both the aluminum and the phosphorus-deficient series became a solid dark purple-red in color and quite brittle in texture. The symptoms were identical with those produced in the beets grown in the untreated acid soil. These solution culture experiments indicate that the lack of phosphorus in a form available for plant utilization is the chief causal factor for the limited growth of beets grown in the previously discussed soil culture experiments, and that there is a connection between a lack of phosphorus and the presence of aluminum.

Drip culture experiments with barley

In order to determine whether phosphorus is precipitated in the soil by aluminum or whether phosphorus is precipitated within the plant tissues, a type of experiment was devised in which a plant could have access to both aluminum and phosphorus but in which these two elements would not be in contact in the culture solution. This would prohibit their mutual precipitation in the culture solution. It was decided to use barley for this experiment as its fibrous root system could be separated into two halves each of which could be immersed in a different culture solution. This would not be possible in growing beets because of the tap root system.

Preliminary drip culture experiments with barley showed that 5 p. p. m. of aluminum in HARTWELL and PEMBER'S (**4**) culture solution was sufficiently toxic to result in a considerable reduction in yield. When barley grown in the standard culture solution lacking phosphorus was compared with that grown in the presence of aluminum a decided similarity in symptoms was evident. In both cases the roots were dwarfed, thick, and showed brownish discolorations. Under both conditions top growth was about equally reduced and a reddish discoloration appeared at the base of the stem.

In the experiment in which the root system was separated into two portions, barley seedlings one week old were planted on the contacting edge of two adjacent containers, one-half the root system being placed in sand in each container. Each half of the root system in its container received a different culture solution by a constant drip from a reservoir. By thus dividing the root system it was possible, wherever desired, for the plants to have access to both aluminum and phosphorus in separate containers. Keeping these

two elements separate prevented aluminum from precipitating the phosphorus in the culture solution.

The various culture solution modifications used are shown in table II.

At the end of an eight weeks' growing period the plants were harvested in three fractions, consisting of the tops and each half of the root system,

TABLE II

COMPARISON OF PHOSPHORUS AND ALUMINUM CONTENT OF BARLEY GROWN IN VARIOUS CULTURE SOLUTIONS

TREATMENT	PLANT FRACTION ANALYZED	YIELD DRY WEIGHT BASIS OF NO. 2 AS 100	PHOSPHORUS (DRY MATTER)	ALUMINUM (DRY MATTER)
		%	%	%
No. 1				
Standard culture solution containing 5 p. p. m. of P (same treatment both sides)	Roots left	9	0.384	0.069
	Roots right	10	0.300	0.078
	Roots total	19	0.339	0.074
	Tops	75	0.224	0.021
	Plant, total	94	0.248	0.032
No. 2				
Standard culture solution containing 2.5 p. p. m. of P (same treatment both sides)	Roots left	11	0.280	0.072
	Roots right	9	0.290	0.071
	Roots total	20	0.284	0.072
	Tops	80	0.206	0.022
	Plant, total	100	0.222	0.031
No. 3				
Left: H_3PO_4 5 p. p. m. of P; Right: 5 p. p. m. of Al in standard culture solution lacking P	Roots left	3	0.253	0.296
	Roots right	2	0.257	0.174
	Roots total	5	0.255	0.247
	Tops	13	0.208	0.022
	Plant, total	18	0.221	0.085
No. 4				
Left: Standard culture solution containing 5 p. p. m. of P; Right: 5 p. p. m. of Al in standard culture solution lacking P	Roots left	17	0.294	0.093
	Roots right	8	0.244	0.251
	Roots total	25	0.278	0.144
	Tops	65	0.237	0.019
	Plant, total	90	0.247	0.054
No. 5				
5 p. p. m. of Al in standard culture solution containing 5 p. p. m. of P (same treatment both sides)	Roots left	7	0.230	0.142
	Roots right	7	0.243	0.263
	Roots total	14	0.237	0.203
	Tops	31	0.222	0.024
	Plant, total	45	0.227	0.079

for each treatment used. The yield for each fraction was determined, as well as the amounts of aluminum and phosphorus present. Aluminum was determined by the colorimetric method of WINTER, THRUN, and BIRD (9), and phosphorus by the colorimetric method of TRUOG and MEYER (8). The results of the analyses are recorded in table II.

It is evident that the damage to the plant as a whole is the result of the poor root system caused by the presence of aluminum. In series 3, 4, and 5, where the roots were in contact with aluminum in the culture solution, there is a comparatively large amount of aluminum in the roots. In all cases these roots show the typical symptoms that have been associated with aluminum toxicity. This localization of injury in the roots in immediate contact with aluminum is further indicated by the fact that the tops of the plants from all series show very little difference in aluminum content whether aluminum was added to the culture solution or not.

In order to facilitate the discussion a ratio of the total percentage of aluminum in the roots to the total percentage of aluminum in the tops in comparison with the yields can be used. A direct comparison of the aluminum content of the roots with total yield could be made since, considering the plant as a whole, the great variation in aluminum content of the plants is found in the roots, the aluminum content of the tops being about the same in all cases. Such a comparison would serve to emphasize the fact that the primary effects of aluminum on the plant seem to be localized in the roots with a consequent poor development of the whole plant. However, since the aluminum content of the tops does vary slightly and in view of the fact that small concentrations of aluminum are known to affect the plants experimented with, the ratio method was used. Such a comparison on the basis of ratios is as follows:

SERIES	RATIO OF $\frac{\text{ALUMINUM IN ROOTS}}{\text{ALUMINUM IN TOPS}}$	YIELD
1	3.5	94
2	3.3	100
3	11.2	18
4	7.6	90
5	8.5	45

The reduction in yield in series 4 is decidedly not what might be expected by comparing the ratio of this series with that of series 5. Although the difference between these two ratios is less than unity, the yield in series 4 is twice that of series 5. A comparison of the ratio of series 4 with the ratios of series 1 and 2 shows that that of series 4 is more than twice the magnitude of either series 1 or 2. The reduction in yield in series 4, however, is not

significant. Some explanation for the good growth in series 4 is evidently necessary.

In series 4 there is no opportunity for aluminum to precipitate phosphorus on the outside of the plant since these two elements are in separate culture solutions. In comparing the analyses of the tops and the two sides of the root system it is found that most of the aluminum is in the half of the root system in direct contact with aluminum. This large amount of aluminum in one half of the root system and the low amount in the other half is directly reflected in yields. Those roots not in contact with aluminum have a yield twice as great as those in contact with aluminum. This low yield of roots on the aluminum side can be attributed only to a precipitation within the root tissues as there was no phosphorus in this culture solution. This immediate precipitation of aluminum precludes the translocation of aluminum to other parts of the plant. Consequently the side of the root system not in contact with aluminum functions almost normally and the total plant yield in series 4 is not greatly reduced over that in series 1 and 2.

Although the total phosphorus percentage in series 4 is as high as series 1 and higher than series 2, there is a reduction in yield. This again indicates precipitation of phosphorus within the plant because a high phosphorus content in association with lower yields indicates that some of this phosphorus is unavailable. This is in line with the observations of BURGESS and PEMBER (**2**), who treated acid soils with a heavy application of acid phosphate and found that plants grew well in this soil, although they contained about the same percentages of aluminum as the plants growing in an untreated acid soil. However, the former contained from three to five times as much phosphorus. This leads them to conclude "... that acid phosphate renders soluble aluminum salts non-toxic largely by counteracting their evil effects within the plants themselves after they have been absorbed."

Yields from series 3 were too low to permit accurate analyses, hence this series is not emphasized in reaching any definite conclusions. The low total yield is probably due to the very low yield of roots, so low that the plant probably did not obtain elements essential for its growth from the incomplete culture solutions used. The high aluminum content on that side of the root system in contact with phosphoric acid is difficult to explain, unless it had its source as a contamination in the sodium hydroxide used to adjust the pH of the phosphoric acid. However, a discussion of the results of this series was not omitted as this particular technique may be improved by subsequent investigators.

The results in series 5 may be differently interpreted but it seems quite logical to conclude that these results also support the contention that, in addition to the external precipitation, aluminum renders phosphorus unavailable internally. Although the total percentage of phosphorus in series 5 is

slightly greater than that of series 2 the actual amount of phosphorus present is much less. For example, a yield of 45 gm. dry weight with 0.227 per cent. of phosphorus, as in series 5, makes the actual amount of phosphorus present 0.102 gm. In series 2, having a yield of 100 gm. dry weight and 0.222 per cent. of phosphorus, the actual amount of phosphorus present is 0.222 gm. It could be argued that the plants in series 5 grew as much as the actual limited amount of phosphorus in the plant permitted. This leads to the conclusion that phosphorus was prevented from entering the plant in amounts sufficient to support growth by its precipitation outside the plant by aluminum. This contention might further be supported by showing that the plants in series 5 have a smaller total percentage and a smaller actual amount of phosphorus in them than series 1, although the amount of phosphorus in the culture solution was the same in both cases. This line of reasoning overlooks the consideration that if phosphorus were precipitated in the culture solution by aluminum, thus cutting down its absorption, it would follow that aluminum also would be in a form that would not readily pass through the plant membranes. The analyses, however, indicate that the roots had absorbed appreciable amounts of aluminum. In fact, the actual amounts of aluminum in the root system of series 5 are in the same range of magnitude as the amount of aluminum in the right side of the root system in series 4, where it would be impossible for precipitation to occur in the culture solution, as, in this latter instance, aluminum, but no phosphorus, was present in the culture solution. It seems logical to conclude from these results that, in addition to external precipitation, aluminum causes injury, even in this series, by precipitating phosphorus within the tissues of the plant where it causes a phosphorus deficiency which is reflected in the poor growth of the barley.

Most workers are of the opinion that superphosphate renders aluminum non-toxic because of its precipitation in the acid soil or in the culture solution. BURGESS and PEMBER (**2**), MCGEORGE (**5**), and PIERRE and STUART (**6**) have advanced the opinion that the chief remedial action of superphosphate takes place within the plant where it renders aluminum inactive by precipitation.

The experiments, as recorded in this paper, substantiate the viewpoint that a large application of superphosphate to acid soils overcomes the toxic effects of aluminum by precipitating the aluminum within the tissues of the plant. It is, of course, admitted that the above statement is an inference as far as plants growing under field conditions are concerned. In the field it would be too much of an assumption to attribute the lessening of aluminum toxicity by superphosphate application entirely to internal mutual precipitation. It was because of external precipitation that the divided-root method in the culture solution experiments was used.

Not denying that the beneficial action of superphosphate to plants in the field may be due to some external precipitation of aluminum, it is felt that the majority of workers have overlooked the possibilities of applications of superphosphate such as may supply phosphorus in amounts sufficient to precipitate aluminum internally, and, over and above this, supply sufficient phosphorus for the metabolic processes of the plant.

Summary

1. Beets were grown in flats in an acid soil, and in acid soil separately treated with calcium lactate, superphosphate, and lime. Healthy vigorous plants were obtained with lime and superphosphate and very poor plants in the untreated soil while those with calcium lactate were about midway in their development between these two extremes.

2. Various analyses of the above plants indicate that: (1) pH, as such, is not a limiting factor, as the pH of the acid soil, calcium lactate, and superphosphate flats was about the same; (2) a deficiency of calcium as an essential element cannot be considered as a limiting factor in growth, as all plants contained as much or more calcium than those grown in the limed flat; (3) photosynthesis cannot be considered a limiting factor, as no magnesium deficiency was discovered, and, furthermore, there was an accumulation of both total and reducing sugars in the plants grown in the acid soil; and (4) deficiencies of total nitrogen, of nitrates, and of phosphorus in the plants from the acid soil reveal possible causes of retarded growth in acid soil.

3. Drip culture experiments with beets indicated a phosphorus deficiency as the possible cause of retarded growth and that the presence of aluminum was the probable cause of this deficiency.

4. In order to determine whether aluminum caused a deficiency of phosphorus by precipitation within the tissues of the plant or externally, barley was grown in drip culture solutions wherein the root system was divided, each half being placed in separate containers and receiving different culture solutions. By this method a plant could have access to both aluminum and phosphorus, and precipitation in the culture solution could be avoided.

5. For analysis, the plants from each culture series were fractioned into three parts consisting of each half of the root system and the tops. Determinations for aluminum and for phosphorus were made on each fraction. These figures in conjunction with the yield of the plants as recorded in table II indicate that: (1) damage to the plant as a whole is probably the result of the poor root system caused by the presence of aluminum; (2) aluminum is precipitated immediately in that half of the root system in direct contact with aluminum, whereas the other half of the root system not in contact with aluminum, and the tops, grow practically as well as the check plants; and (3) in that series where each half of the root system is in a culture solution con-

taining both aluminum and phosphorus, internal precipitation evidently plays an important part in retarding growth, since, if the precipitation were external, the fairly large amounts of aluminum and phosphorus found inside the roots of these plants could not be satisfactorily explained.

6. Although the results obtained from these culture solution experiments cannot be used for plants growing under field conditions, it is felt that most workers have over-emphasized the external mutual precipitation of aluminum and phosphorus and have overlooked the fact that the internal precipitation of phosphorus by aluminum evidently plays an important rôle in the poor development of certain plants grown on acid soil. The corrective action of large applications of superphosphate to acid soils is attributed largely to the internal precipitation of aluminum by phosphorus with sufficient phosphorus remaining for the metabolic processes of the plant.

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SEASONAL FLUCTUATIONS IN GROWTH RATES OF EXCISED TOMATO ROOT TIPS

PHILIP R. WHITE

(WITH FOUR FIGURES)

Previous to 1934 ROBBINS (**10, 11**), ROBBINS and MANEVAL (**12, 13**), KOTTE (**6, 7**), CHAMBERS (**1, 2**), MALYSCHEV (**8, 9**), GAUTHERET (**4, 5**), and WHITE (**14–17**) had reported the cultivation of excised root-tips of various species of plants over periods in some cases as great as 5 months. In such of these studies as extended over considerable periods of time, the growth rates showed a more or less continuous diminution, ending in loss of all cultures. Fluctuations in growth rates were apparently random in nature and of no demonstrable significance. In 1934 WHITE (**18**) reported experiments in which two clones of tomato root-tips were successfully grown for one year. Aside from fluctuations due to known variations in environmental factors, the cultures showed not a diminution but an *increase* in growth rate. The improvement in result is probably attributable to a more satisfactory nutrient and technique. The observed increase in growth rate was tentatively attributed to adaptation of the cultured organs to their artificial environment, and it was presumed that the growth rate would ultimately reach a constant level. The present paper proposes to report the behavior of one of these clones (clone C, **18**) over an additional two years.

Since a standard procedure had not yet been developed at the time the cultures were started, the first 10 passages were of irregular length and were maintained under a variety of experimental conditions. Passages 11–23 were poor for reasons discussed elsewhere (**18**). With the 11th passage a standard procedure (**18**) was adopted which has since remained essentially unchanged. The present paper, then, records the behavior of this clone through 177 passages. Of these, 167 have had a duration of one week each and have been maintained under as uniform conditions as were available in an ordinary laboratory room. In passages 11–52, 100 roots were grown in each passage so that each growth rate is the mean of 100 measurements. In all passages subsequent to 52 this number was reduced to 25.

Figure 1 shows the growth rates of this clone of cultures during 177 passages. The records for the last 125 weeks have not been previously published. The line AA′ represents the average growth level for the entire 177 weeks, 6.2 mm. per culture per day. The measured total increment recorded, exclusive of branches, is about 30,000 times the magnitude of the tissue fragment with which the clone was started. The clone is being maintained and, after 3 years' cultivation *in vitro,* is still in excellent condition.

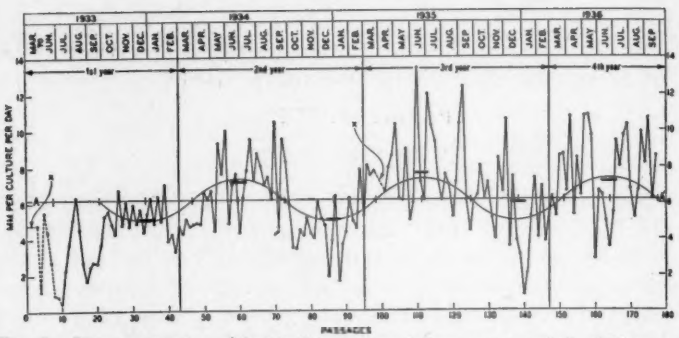

FIG. 1. Increment rates of isolated tomato root-tips over a period of 3½ years. The average weekly increments are represented by the zigzag line. The line AA′ represents the average increment level for the entire period. The heavy lines at values of the sine curve $\theta = +1$ and $\theta = -1$ represent the average increment rates of each 26-week period beginning on September 21 and March 21, each period corresponding to a half-wave of the curve. Measurements were not recorded for passages 1, 2, 99, and 100 (marked "x").

In figure 1, the observed fluctuations in growth rate are rather uniformly grouped around the mean level AA′. The clone has, therefore, after the first period of adaptation, settled down to a uniform growth level, as was anticipated. Nevertheless, analysis of the growth curve shows it to contain two types of deviation from the mean. Random deviation from week to week is the most evident component. It seems probable that inequalities in the amount of trauma, inflicted in the manipulations of excision and transfer, may affect the subsequent growth rates. It has also been noted that the position of the explant in the parent culture—whether taken from the tip, from a branch near the center of the culture, or from the base—may affect its subsequent growth rate. There are also marked differences in the vitality of individual branches at the same level, which may be evident in the color of the meristematic region. Doubtless there are other differences which are not visible. These, and probably other factors contribute to the variability of the cultures. The range of this deviation can be greatly reduced by the use of large numbers of cultures, as is evident in passages 27 to 39 where 100 cultures were maintained. It might conceivably be eliminated by the use of sufficiently large numbers or by more careful selection than has been practiced in preparing this routine stock.

When these week to week deviations are smoothed out by averaging together the indices of several passages, there still remains a second component of the curve which shows a regular cyclic fluctuation with maxima lying just a year apart. The horizontal blocks in figure 1 indicate the positions of such means when groups of 26 passages beginning with passage 23 are so averaged. If a flattened sine curve be drawn with AA′ as its axis, the wave

crests spaced 52 weeks apart, with minima (sine $\theta = -1$) falling on December 21, the curve will be seen to coincide rather closely with the observed growth rates, strongly suggesting that these fluctuations must follow some simple law related to the seasons.

Yearly cycles are, of course, common in the plant kingdom. In some cases these are obviously the result of the annual cyclic fluctuations of light and temperatures resulting from the alternation of seasons, and disappear under controlled environmental conditions. In others, *Asparagus medeoloides* Thunb., for example, the cycle is inherent and so firmly established in the make-up of the plant that in the northern hemisphere it continues to undergo its dormant period in May to September in spite of a completely inverted environmental cycle, and years of cultivation in the north will not alter this behavior pattern. It has seemed desirable to determine whether the cycle observed in these root-tips can be shown to be correlated with uncontrolled factors in the environment, or if it may be inherent.

The cultures in question were maintained under as uniform conditions as were available for large numbers. But, since they were kept in a laboratory room where other work was being carried on simultaneously, they were subject to two variables known to be only partially controlled and perhaps to other unknown ones. These two variables were light and temperature, both of which undergo seasonal cyclic fluctuations.

The cultures under consideration were at no time exposed to direct sunlight but, since they were placed in a well-lighted room, they received diffuse light of greater intensity and longer daily duration in summer than in winter. ROBBINS and MANEVAL (**13**), WHITE (**15**), and FELBER-PISK (**3**) had reported moderate illumination to be beneficial to similar cultures of root-tips of other plants, so that light might conceivably have been an important factor in determining the observed fluctuations. However, the studies of these authors were all of too short duration to be of undoubted significance. MALYSCHEV, moreover, was unable to substantiate this conclusion (**9**). To test the question more thoroughly, a double-walled box was built of opaque matte-black cardboard, large enough to hold 25 cultures. It was so arranged as to allow free circulation of air, but light was completely excluded. Fifty cultures were prepared from passage 143, clone C. Twenty-five of these were placed in the dark box and set alongside of the other 25 which represented passage 144 of figure 1. Both sets of cultures were then transferred at weekly intervals for 10 weeks, being measured as usual at the beginning and end of each passage. Measurements and transfers of both "dark" and "light" cultures were made by daylight. The "dark" cultures were thus exposed to diffuse light for somewhat less than an hour each week, that is, about 0.5 per cent. of the elapsed time, while the "light" cultures were similarly exposed approximately 100 times this length of time (50 per cent.

of the elapsed time). The growth rates obtained are shown in figure 2. The mean difference in growth rates between the two sets for the entire 10 weeks was only 0.6 per cent., in favor of the "dark" cultures. This is too small a difference to be significant. It is worth noting, however, that had only passage 151 been measured, the result—a 40 per cent. increase of the "light" cultures over the "dark" ones—would have been in agreement with the findings of the earlier authors cited above, while observation of passage 152 alone would have led to an entirely contrary conclusion. The danger in

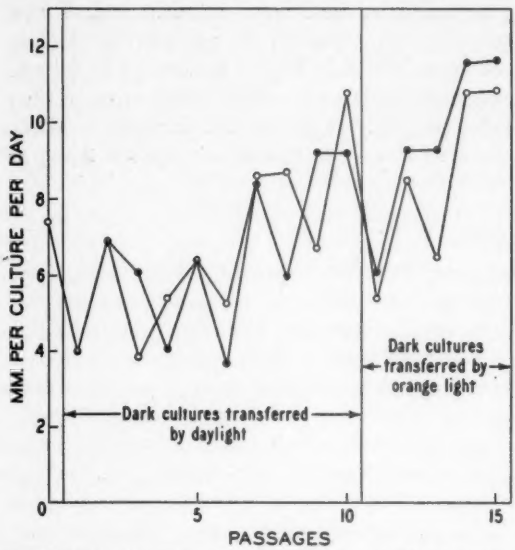

FIG. 2. Growth rates of cultures grown in diffuse daylight (open circles) and in darkness (solid circles) for 15 weeks.

drawing conclusions from single passages is thus evident. To make the result doubly sure, transfers were made for an additional 5 weeks, the "dark" cultures being transferred by the light of an orange Wratten Safe Light no. 0, without other exposure at any time. Throughout these 5 weeks (fig. 2), the cultures grown in the dark were consistently somewhat better than those provided with diffuse daylight. Since 15 weeks of greatly reduced illumination did not appreciably reduce the growth rates of these cultures nor cause any visible abnormalities in their behavior (fig. 3), it may safely be concluded that light is neither essential nor significantly beneficial to isolated tomato root-tips. Seasonal variations in illumination can, therefore, be ruled out as a factor in determining the cyclic variation in growth rate recorded in figure 1.

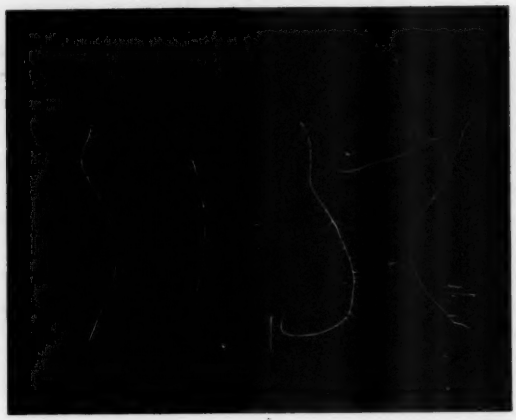

FIG. 3. Roots grown in diffuse daylight (two at left) and in continuous darkness except for the time required in transferring (two at right). The photograph was taken at the end of the 15th weekly passage under these conditions. × 0.6.

The room in which the cultures were maintained was thermostatically controlled as to *minimum* temperature, so that the temperature never fell below 20° C. Since, however, no provision was made for cooling when the outside air temperature exceeded this figure, the room temperature sometimes rose to 32° C. WHITE had earlier shown (**15**) that isolated wheat roots are sensitive to variations in temperature, growing best at about 27° C., so that this also might have been an important factor in determining the observed cycle.

Had light proved to be a significant factor in determining growth rates, the exact evaluation of temperature effects would have been difficult, since, in order to obtain comparable results, it would have been necessary to maintain a uniform light intensity at all temperatures studied. Since, however, variations in illumination over a rather wide range of intensity are without significant effect, this factor can be ignored in temperature studies so long as the light intensity is kept low. To test the effects of temperature variations, constant temperature ovens providing no illumination and controllable to ± 2° were used for temperatures 5°, 8°, 10°, 15°, 20°, 25°, and 28° C. Temperatures of 30°, 31°, 32°, 33°, 35°, and 40° C. were provided in a water bath which admitted a low intensity of light to the cultures and was controllable to ± 0.5°. Twenty cultures were grown at each temperature, and measurements made at the end of one week. The mean growth rates for single passages at these temperatures are shown in figure 4. They are to be compared with those shown in figures 2 and 3 in the author's earlier work on wheat root-tips (**15**). The optimum at 30°—slightly higher than for

wheat—is extraordinarily sharp, an increase in maintained temperature from 28° to 30° causing a 20 per cent. increase in growth rate, while a further temperature increase from 30° to 31° caused a 30 per cent. drop in growth rate. Unlike variations in illumination, variations in temperature are obviously of great importance in determining growth rates.

The range of temperatures to which the cultures, whose growth rates are shown in figure 1, were subjected was, as has been said, from 20° to 32° C. As shown in figure 4, this is sufficient to determine a range of mean growth

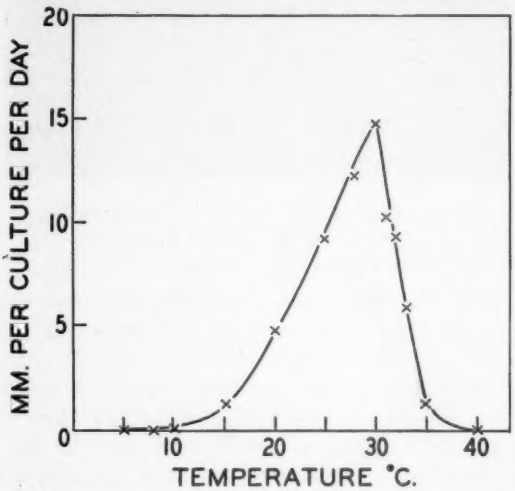

FIG. 4. Graph showing the relation between mean growth rate and maintained temperature, recorded over a single passage.

rates from 5 mm. per culture per day (at 20°) up to 15 mm. per culture per day (at 30°) and then back to 9 mm. per culture per day at 32°. Cultures grown at 32° were of poor color, often misshapen, and obviously in unsatisfactory condition. If the growth rates recorded in figure 4 be compared with the mean growth rates for the various seasons, as shown in figure 1, it will be seen that the growth rate for 20° C.—5 mm. per culture per day—corresponds very closely to the rates obtained during three consecutive winters—5.1, 5.1, 6.0—when, owing to low outdoor temperatures, it was possible to maintain the room temperature at 20–22°. The growth rate for 24°, which may be considered an average room temperature for summer,—8 mm. per culture per day—corresponds closely to the rates observed during the three summers covered by the record—7.2, 7.7, 7.2. Moreover, the maximum growth rate recorded in figure 4—15 mm. per culture per day at 30° C.—differs only slightly from that recorded in figure 1, 13.4 mm. per

culture per day in June, 1935, at a time when the mean temperature of the culture room may easily have approached such a value.

From the figures presented above it is evident that variations in temperature of the range to which the cultures under consideration are known to have been subjected, a range characteristic of a moderately well controlled laboratory, are quite sufficient to explain the cyclic fluctuations recorded in figure 1. Whether this is, as the data seem to indicate, the sole explanation or whether, when this variant is eliminated by more careful control of the temperature, there will remain a residual cyclic variation which is inherent, must await further study over an additional period of a year or more. The record presented here, however, serves to emphasize what has been repeatedly noted before, that growth which is sufficiently active for experimental purposes and which is reproducible can be obtained with root-tip materials only when all cultural conditions are rigidly controlled. Sensitivity to small variations in temperature and to slight variations in the concentrations of many of the nutrient ions must be taken into consideration in the interpretation of all results. The importance of these particular variables can hardly be over-emphasized; but as is evident from the results with various intensities of illumination, there also exist variables which are not important.

Summary

The growth rates of a clone of isolated tomato root-tips cultivated *in vitro* and measured at weekly intervals for a period of 3 years show a random fluctuation around a mean due to variations in the behavior of the individual cultures. In addition, these growth rates show a cyclic fluctuation correlated with the seasons of the year. Investigation of the effects of light *vs.* darkness showed that seasonal fluctuations in illumination are a negligible factor in producing this cycle. Investigation proved, however, that such cultures are very sensitive to temperature differences and that the observed fluctuations in temperature are sufficient to account for the observed seasonal variations in growth rate. It will probably be necessary to control the room temperature more accurately than has been done in the past if uniform cultures are to be maintained throughout the year.

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STRUCTURAL CHANGES PRODUCED IN LEAF TISSUE OF SOY BEAN PLANTS BY IRRADIATION OF THE DRY SEEDS WITH SOFT X-RAYS

THEO. P. LONG AND H. KERSTEN

(WITH EIGHT FIGURES)

Introduction

In a recent paper NOGUCHI (**2**) described histological studies made on the structure of Russian sunflower leaves grown from seeds irradiated at the stage of incipient germination, with hard, or short wave-length, x-rays. This paper describes a similar study in which the seeds used were dormant soy beans and the radiation was soft, or long wave-length, x-rays generated by a gas x-ray tube (**1**). Although it is not the purpose of this paper to compare the two types of radiation with regard to their ability to produce deformed plants from irradiated seeds it may be said that if a given quantity of radiant energy in the x-ray region here considered should strike the surface of a seed, a greater part of it would be absorbed by the seed if the radiation were of long wave-length than if it were of short wave-length. If the extent of the deformity produced in the plants grown from x-irradiated seeds depends on the quantity of energy they have absorbed, then one would expect the longer wave-length to produce the greater deformity, if the incident quantities of energy were alike.

Method

Wilson black soy bean seeds which had been stored under ordinary conditions of temperature and humidity for about a year were used. They had therefore acquired the moisture content normally associated with dormant seeds. These were placed 8 cm. from the focal spot of the x-ray tube and were irradiated for periods from 10 to 80 minutes with the x-rays from the tube mentioned, when it was operated at 20 peak kv. and 10 m.a. Under these conditions the shortest wave-length in the beam of x-rays was about 0.6 Å., while the most intense part of the beam was the K_α line of copper (1.54 Å.). The beans were then planted in ordinary soil in a greenhouse, together with unirradiated ones for controls. The irradiated seeds produced deformed plants, of which figure 1 is an example, while the unirradiated seeds produced normal plants. Those which were irradiated for the longer periods of time produced plants exhibiting similar deformities to a greater degree.

Results

After an examination of the outward appearance as well as the corresponding sections of a large number of deformed leaves, it was found that almost all of them fell into one of the following classes:

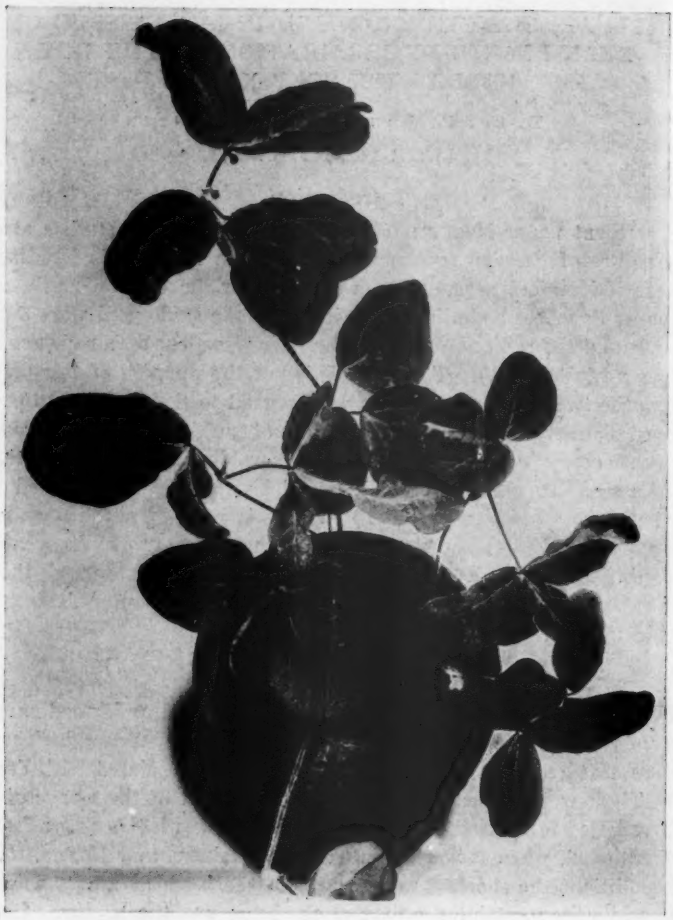

FIG. 1. Soy bean plant with injured leaves.

1. Normal leaf (fig. 2). The epidermal cells are of uniform rectangular shape and form a fairly smooth leaf surface. The palisade layer is composed in most cases of two layers of cells placed evenly and having only a small amount of space around each cell. The chloroplasts are arranged just inside the cell wall and are spaced evenly. In the spongy parenchyma layer the cells show an irregular pattern in cross-section and have the chloroplasts arranged irregularly. The spongy tissue extends all the way to the epidermis.

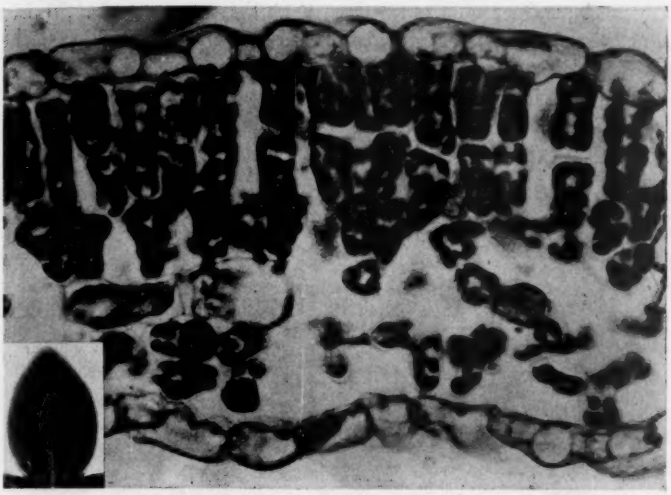

FIG. 2. Normal leaf.

2. Leaf with numerous small light spots (fig. 3). These spotted areas are caused by the failure of the upper layer of palisade cells to develop

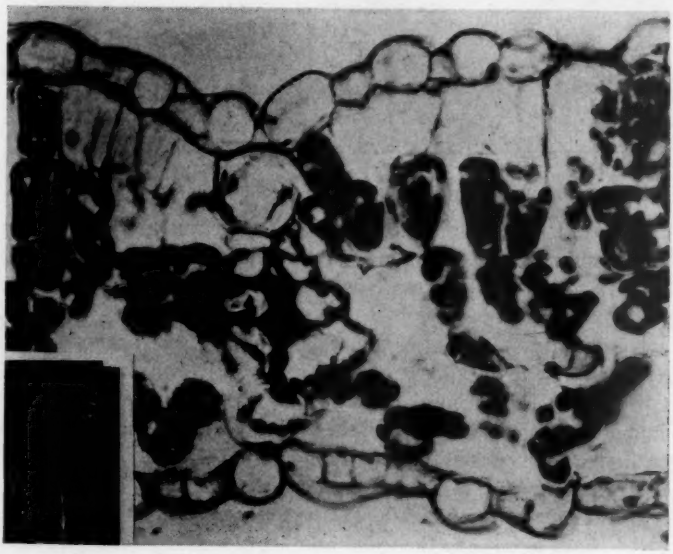

FIG. 3. Leaf with numerous small white spots.

normally and produce chloroplasts, so that these areas are light in color. Sometimes the second layer of palisade cells is also affected. Neither the epidermis nor the spongy tissue is noticeably changed.

3. Leaf with large light-grey areas, found mainly on the second and third sets of leaves (fig. 4). In this case the palisade cells appear to be

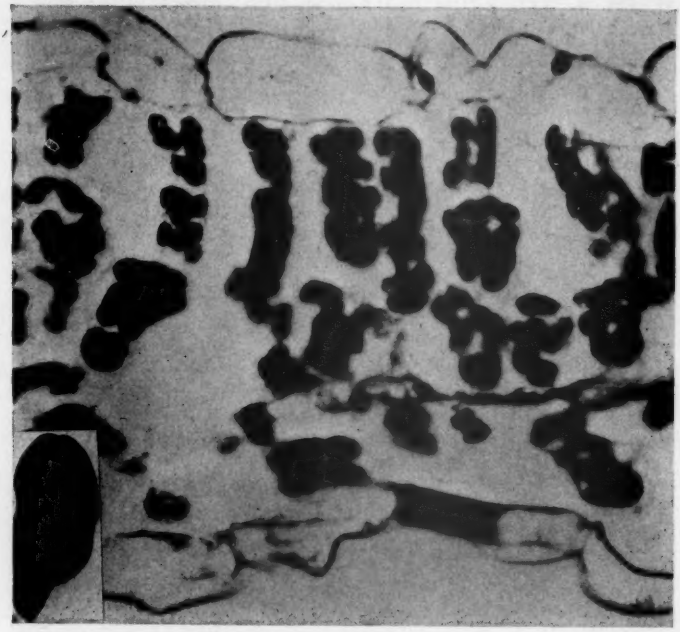

FIG. 4. Leaf with light-green area.

normal but are spaced farther apart. In some leaves there are fewer than the normal number of chloroplasts in the cells. The chloroplasts in the cells of the spongy parenchyma layer are arranged irregularly, sometimes clumped together.

4. Leaf with small dark-green areas which appear normal except for color (fig. 5). (The dark-green areas are difficult to show in photographs of the leaves.) This injured condition seems to be due to an unusually large number of chloroplasts containing an excessive amount of chlorophyll in both the palisade cells and in the spongy tissue.

5. Leaf with large dark-green areas often raised and crinkled (fig. 6). In this kind of tissue the palisade cells are closely packed together and the cells seem to have more than the usual number of chloroplasts. The palisade

FIG. 5. Leaf with small dark-green areas.

cells are usually somewhat elongated and extend into the spaces belonging to the spongy parenchyma. The spongy tissues are smaller in volume, and the cells adjacent to the lower epidermis are often arranged as a layer of short palisade cells containing many chloroplasts. The epidermis, especially the upper layer, is usually irregular with more rounded and otherwise oddly shaped cells. The leaves from which these sections were made received illumination from above, while growing, just as those exhibiting the other types of deformity. In both types 4 and 5, some of the cells contain chloro-

FIG. 6. Leaf with large dark-green areas.

plasts which are of a dark blue-green color suggesting a possible abnormal percentage of chlorophyll 'a' and 'b.'

6. Leaves with white areas, either albino patches on the green leaf, or an entirely white leaf (fig. 7). In the albino leaves the palisade cells are

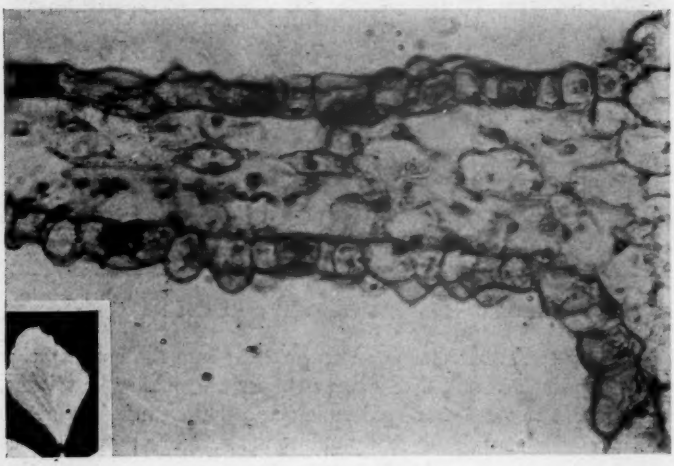

FIG. 7. Albino leaf.

entirely absent in the specimen examined. Only one plant exhibiting marked albino characteristics was grown (fig. 8). It grew from seed which had been irradiated 10 minutes at 35 peak kv. and 20 m.a., placed 30 cm. from the focal spot. The albino characteristics may have been due to factors other than the x-irradiation but sections of its leaves are interesting when compared with the types of injury which can be produced at will. The spongy tissue seemed normal except for the absence of almost all the chloroplasts, while those present contained little or no chlorophyll. The epidermis was composed of rounded and oddly shaped cells. The veins were abnormally near each other and stood out as ridges. The intervascular part of the leaf was about 60 μ thick compared with about 160 μ for a normal leaf. The tissues were very difficult to stain and photograph.

7. Leaf with areas completely absent. (Several examples are shown in fig. 1.)

The authors wish to thank Dr. J. HOBART HOSKINS, and Dr. F. M. TURRELL of the Botany Department for their interest and assistance.

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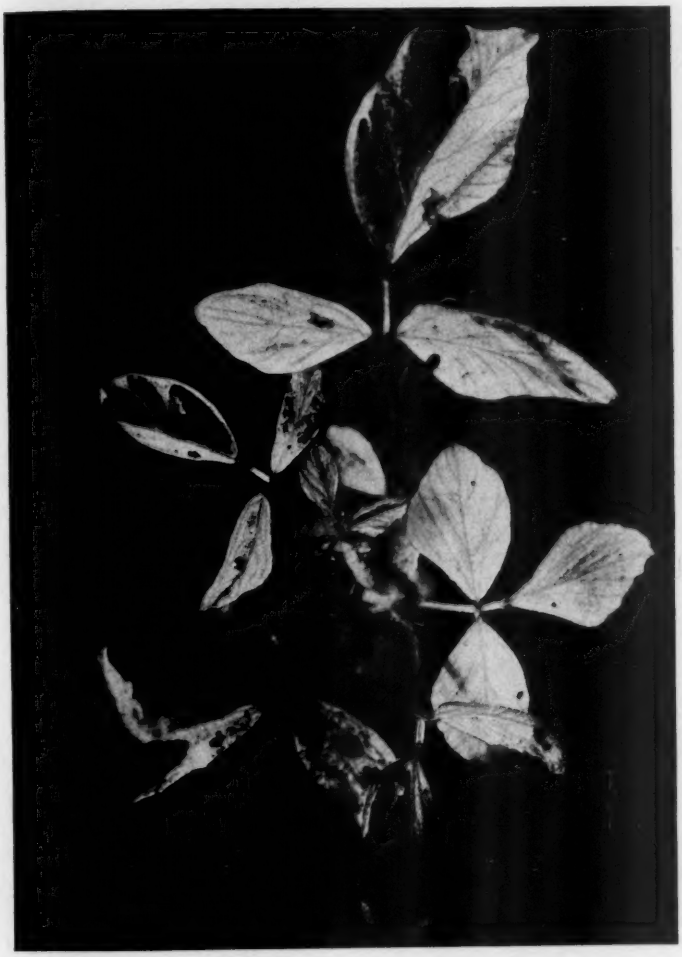

FIG. 8. Plant showing marked albino characteristics.

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NUTRITIONAL STUDIES ON LOBLOLLY PINE

RUTH M. ADDOMS

(WITH TWO FIGURES)

Introduction

The investigation herein reported was undertaken as part of a cooperative project on the biology of forest trees at Duke University. It represents an attempt to add a contribution to knowledge of the mineral nutrition of certain forest trees, in the hope that ultimately it may be possible to correlate the findings with studies of soil composition and with the local distribution of these tree species. Loblolly pine (*Pinus taeda* L.) was selected as a species that is relatively cosmopolitan, and yellow poplar (*Liriodendron tulipifera* L.) as a species that is relatively specific in environmental requirements. The present paper is a preliminary report on loblolly pine only.

For the purposes of this investigation, sand cultures were regarded as the most desirable method. Of the extensive work with sand cultures by many investigators within the past few years, little has been concerned with woody plants, and very little with forest trees. MITCHELL'S (4) experiments on Scotch pine and white pine in the Black Rock Forest represent the most fruitful work of the kind that has been done with forest trees.

Experimental methods

Loblolly pine was grown in sand cultures for periods as long as 29 months. The first experiment was started in February, 1934, when 50 seedlings, 8 months old, were transplanted from a sand-peat mixture to washed white quartz sand in ordinary 10-inch unglazed pots. Of these seedlings 30 were transplanted in January, 1935, to sand in 3-gallon glazed crocks with drainage at the bottom. Nutrient solutions were added at first twice and later three times per week; at all other times the moisture content was maintained by the addition of distilled water. From time to time individual plants were removed from the series for study. Four of the 22 plants that remained on July 14, 1936, are shown in figure 1.

Other series were grown for shorter periods of time, employing similar methods except that glazed self-draining pots were used from the start.

Another series of loblolly pine was grown in sand culture for 14 months under slightly different conditions. The same type of crocks and the same solutions were used, but the solutions were applied daily by a drip-method of renewal. Addition of nutrients was not continuous, but 10 pots set up in series received each day 18 liters of solution, which dripped from capillary tubes. The arrangement of apparatus is shown in figure 2. In this series, one

FIG. 1. *Pinus taeda* L. grown for 29 months in sand culture. For two trees on left, nitrogen was supplied as calcium nitrate; for two trees on right, as ammonium sulphate.

FIG. 2. Drip-cultures at the end of 14 months. Capacity of each carboy is 18 liters. Uniform rate of drip is maintained by means of bottles that serve as constant-level reservoirs. Solution reaches individual cultures by dripping from capillary tubes. For series on left, nitrogen was supplied as calcium nitrate; for series on right, as ammonium sulphate.

set of 10 pots received its nitrogen from calcium nitrate, the other set from ammonium sulphate.

Since the experiments were primarily exploratory in nature in all series, the composition of solutions was varied from time to time in an attempt to determine the optimum conditions for growth. Concentrations of salts were varied as well as the hydrogen ion concentration, the latter by means of the addition of NaOH and H_2SO_4. The composition of two of the solutions that were successfully employed is given in tables I and II.

TABLE I

COMPOSITION OF SOLUTION A

SALT	GRAMS PER LITER	CONCENTRATION IN P.P.M.			
	gm.	*p.p.m.*		*p.p.m.*	
$Ca(NO_3)_2 \cdot 4H_2O$	1.148	Ca	195	N	136
KH_2PO_4	0.643	K	184	P	146
$MgSO_4$	0.283	Mg	57	S	76
H_3BO_3	0.001	B	0.20		
$MnSO_4$	0.001	Mn	0.40		
$FeCl_3$	0.002	Fe	0.70		

TABLE II

COMPOSITION OF SOLUTION B

SALT	GRAMS PER LITER	CONCENTRATION IN P.P.M.			
	gm.	*p.p.m.*		*p.p.m.*	
$(NH_4)_2SO_4$	0.734	N	156	S	156
KH_2PO_4	0.870	K	249	P	198
$MgSO_4$	0.167	Mg	33	S	45
$CaCl_2$	0.324	Ca	117	Cl	204
H_3BO_3	0.001	B	0.20		
$MnSO_4$	0.001	Mn	0.40		
$FeCl_3$	0.002	Fe	0.70		

Results and discussion

The several experiments, extending over periods of varied lengths up to 29 months, proved to the satisfaction of the writer that (1) loblolly pine can be grown successfully in sand culture, and that (2) it can utilize nitrogen in the form of either nitrates or ammonium compounds. These two points will be discussed in some detail.

The pine trees grown in sand culture compared favorably with trees grown in soil as to size and general appearance, with the exception of color

as discussed below. One difficulty that was experienced in sand culture, however, was the inequality of growth of the different individuals in a series. All of the plants grew, but the amount of growth varied greatly. When the number of individuals in a series must be limited, this factor is important. MITCHELL (4) refers the difference in his seedlings to difference in seed weight, and cites correction factors. In the present experiments an attempt was made to obviate the difficulty by using seedlings several months old and selecting them for uniformity of size and general appearance. The results indicate, however, that such selection is not sufficient. Figure 2 shows that the size of plants after several months of identical cultural treatment varied greatly. This difference was obviously the result of differences in plants and not of differences in treatment. In experiments with plants that can be propagated vegetatively, it would probably be better to work with rooted cuttings than with seedlings.

A second difficulty was the yellowing of leaves that was apparent from time to time in both nitrate and ammonium series. Usually it showed during the first few months after the seedlings were set out, and in most instances the plants subsequently recovered. The yellowing was much less noticeable in the drip cultures. In the light of MITCHELL'S (4) experience it seems reasonable to suppose that the cause was insufficient nitrogen. Mitchell states that the optimum concentration of nitrogen is 300 p.p.m., whereas in the solutions listed above, the concentration of nitrogen was only about half of that. In his experiments, however, the nutrient solution was added only twice during the 15 weeks of the experiments, whereas in the present experiments nutrient solution was added two or three times a week, and in the series of drip-cultures, every day. It is obvious, then, that the total amount of nitrogen supplied to the plants was considerably more than the concentrations indicate, in comparison with those of MITCHELL'S solutions. However, the fact that the pines of the drip-series showed much less yellowing than the others lends some evidence to the theory that the abnormality was caused by an insufficient supply of nitrogen. It was obviously not caused by deficiency of boron, manganese, or iron; iron was added in several forms and in several concentrations during the experiments, without apparent effect. Alteration of the hydrogen ion concentration from time to time during the course of the experiments had no appreciable effect on the yellowing. As stated above, most of the plants recovered and developed a healthy green color. Similar yellowing has been observed in soil cultures, and occasionally in the field.

Some of the seedlings developed a peculiar twisting and curling of the leaves that produced an appearance of knotting. This was observed in some but not all plants of every series, and in some plants more generally than in others. Eventually the leaves straightened and assumed a normal appearance. The abnormality was not restricted to these experiments, but was ob-

served in even more acute form in some soil cultures that were being conducted by another worker in the adjoining greenhouse. It has since been observed occasionally in pine trees growing in the field. The first explanation that suggested itself was that the sheath of the fascicle had become dry and hard, with the result that the normal elongation of leaves was mechanically restricted. In order to test this theory, several plants were placed for several weeks under bell jars, so that the humidity in which they were growing was greatly increased. Under this treatment leaves were as "knotted" as ever, disproving the theory.

These experiments showed conclusively that loblolly pine is capable of utilizing nitrogen in the form of either nitrates or ammonium. Alteration of the acidity over a wide range showed the best development with calcium nitrate as the source of nitrogen when the solution was decidedly acid (3.8 to 5 pH); with ammonium sulphate as the source of nitrogen when the solution was more nearly neutral (6 pH). The observations are entirely in keeping with those of TIEDJENS (**6**), TIEDJENS and BLAKE (**7**), TIEDJENS and ROBBINS (**8**), NIGHTINGALE (**5**), and DAVIDSON and SHIVE (**2**) on apple, peach, and other plants. It is altogether probable that under field conditions in the Piedmont of North Carolina loblolly pine receives most of its nitrogen in the form of ammonium compounds, for little nitrification occurs. In many forest stands of loblolly pine, as determined by COILE (**1**), the hydrogen ion concentration of the A horizon varies from 5.4 to 6.5 pH.

Although mycorrhiza were present when the seedlings were set out, they did not develop to any considerable degree in sand cultures. Field-grown plants show long white roots that are free from fungous infection and short, stubby, much-branched lateral roots that are infected with a tight mass of mycelium that penetrates the epidermis. Pines of the several experimental series also showed long white roots and short, branched lateral ones; however, the latter were not nearly so stubby as those of field-grown plants and microscopic observations showed no trace of fungous infection—any mycelium that was present was entirely external. Since rapid and apparently healthy growth was obtained in sand culture, it would seem likely that in field-grown loblolly pine the occurrence of mycorrhiza is incidental rather than highly beneficial.

The root systems developed in sand culture are worthy of mention, as their distribution is in rather striking contrast to that of loblolly pine roots under field conditions. In nature, most of the absorbing roots occur in the uppermost layers of the soil. In sand culture, however, the root systems practically fill the pots, and the absorbing roots are much more generally distributed. It is possible that the difference is attributable to the difference in aeration, and that the superficial character of the root systems of loblolly pines in many forest soils is caused by the deficiency of aeration in underlying horizons.

During the course of the present experiments, the trees were not dormant through a long period of time, such as the winter. In all of the series they showed during a year several periods of growth and several periods of dormancy, but never a long-continued winter dormancy. It is altogether possible that this condition is attributable to illumination. Although the pines were not intentionally given a longer photoperiod, other experiments in adjoining greenhouses were lighted regularly. It is possible that sufficient light reached the pines to prevent winter dormancy. WITHROW and BENEDICT (9) have shown that for certain plants an intensity of 0.3 foot candle is sufficient to produce a response. KRAMER (3) has demonstrated that it is possible to keep loblolly pines growing actively throughout the winter under greenhouse conditions.

In the light of these experiments the writer feels justified in pursuing the study of mineral deficiencies in loblolly pine by the use of sand cultures, employing either nitrates or ammonium salts as sources of nitrogen.

Summary

1. Loblolly pine seedlings were grown successfully in sand cultures for periods up to 29 months.

2. They proved capable of utilizing nitrogen in the form of either nitrate or ammonium—the former more successfully in acid reaction, the latter more successfully in a more nearly neutral reaction.

3. The seedlings showed great individual variation in rate of growth even though they were selected on the basis of uniformity of size.

4. The root systems were deeper than those that are usually developed in the field.

5. Mycorrhiza were not formed.

6. Many plants of both the nitrate and the ammonium series showed, at times, a yellowing of leaves that may have been caused by an insufficient quantity of nitrogen. Apparently similar conditions were observed in soil cultures and to a lesser degree in the field.

7. Plants were grown throughout the year without long-continued periods of dormancy. This continuous activity may have been associated with a photoperiod longer than that of winter days.

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EFFECT OF ETHYLENE CHLORHYDRIN AND THIOUREA ON *ELODEA* AND *NITELLA*[1]

BEATRICE MARCY

Introduction

Ethylene chlorhydrin and thiourea were found by DENNY (**4**) to have the power of breaking the rest period of freshly-harvested potato tubers. DENNY (**8**), GUTHRIE, DENNY, and MILLER (**6, 9**), and MILLER (**11, 12, 13**) were unable to find any close correlation between the power of these chemicals to produce early sprouting and their effect upon respiration, glutathione or sulphydryl content of the tissues, or enzyme activity, which might explain how dormancy is broken. The purpose of this study was to see if ethylene chlorhydrin and thiourea produced any change in the rate of the streaming of protoplasm, a change which might indicate their effect upon protoplasm itself, and so throw some light upon the manner in which dormacy is broken by them.

Method

The plants used were *Nitella* sp., originally collected near Northampton, Mass., and *Elodea canadensis gigantea* (*E. densa*), obtained in January, 1936.

The chemicals, ethylene chlorhydrin and thiourea, were obtained from the Research Laboratory of the Eastman Kodak Company. Distilled water from a zinc still was used for all solutions. The concentrations of the solutions were 10 to 0.025 gm. per liter. Check tubes containing distilled water, but no chemical, were included with each set of experiments. Some additional measurements were made for each species in tap water.

For each test, 30 cc. of each solution were used. About four inches of the terminal portions of plants of *Elodea*, and of filaments of *Nitella* were placed separately in cotton-stoppered test-tubes containing the solution, and arranged so that all were equally lighted from an adjacent window. The temperature, at the time of taking the readings, varied at most 5° C. At 2-, 24-, and 48-hour intervals, one leaf was detached from each *Elodea* shoot. This leaf was always from among the lower leaves of the terminal bud. Rate of streaming in millimeters per second was measured with a standardized ocular micrometer, and a standardized metronome. From each leaf ten readings were made, from adjacent cells at the base of the leaf, near the midrib. For *Nitella*, readings were taken, as much as possible, from cells similar in size and age. Thus, there were tested two chemicals, each in ten dilutions, used

[1] This paper was presented before the New England Section of the American Society of Plant Physiologists at Storrs, Connecticut, May, 1936.

on two species, and treated for three different lengths of time. Each reading was checked by ten counts, and the entire experiment was done five times.

Experimental results

Both ethylene chlorhydrin and thiourea, in concentrations of less than 1 gm. per liter, increase the rate of streaming of the protoplasm of *Elodea* and *Nitella* after these plants have been treated for 2, 24, or 48 hours. A solution containing 50 gm. per liter of either chemical causes the death of the cells of both plants after 24 hours' treatment (tables I–IV).

When *Elodea* is treated with ethylene chlorhydrin for either 24 or 48 hours, the point of maximum streaming falls at a concentration of 0.1 gm. per liter or less (table I). Concentrations higher than this cause a decrease in the rate of streaming, movement being completely stopped at 10 gm. per liter. However, with only 2 hours' treatment the maximum rate is at a concentration of 10 gm. per liter, and the lower concentrations have not affected the rate of streaming in any significant way (table I). It appears that in 2 hours enough chemical to effect the protoplasm is absorbed only from the 10 gm. per liter solution.

TABLE I

ELODEA TREATED WITH ETHYLENE CHLORHYDRIN

CONCENTRATION IN GRAMS PER LITER	RATE OF STREAMING*		
	2 HOURS	24 HOURS	48 HOURS
gm.	*mm./min.*	*mm./min.*	*mm./min.*
10.000	0.5388†	0.0000	0.0000
0.750	0.2256	0.2880	0.2686
0.500	0.2484	0.3384	0.2460
0.250	0.2868	0.3805	0.3433
0.100	0.2797	0.4380†	0.3900
0.075	0.2856	0.3960	0.3949†
0.000	0.2616	0.2412	0.2208
Maximum percentage of increase	100.59	81.6	78.84

* Each figure represents the average of 50 readings.

† Maximum rate recorded.

When *Elodea* is treated with thiourea for 24 or 48 hours, the effect is similar, except that streaming is not altogether stopped by a solution of 10 gm. per liter (table II). The maximum rate is induced by a concentration of 0.1 gm. per liter. The rate, after 2 hours of treatment, is greatest at a concentration of 0.5 gm. per liter, but the difference between the effects of the different concentrations is not marked. Again, the rates of streaming in various concentrations after 24 and 48 hours are very similar for the two chemicals.

TABLE II

ELODEA TREATED WITH THIOUREA

CONCENTRATION IN GRAMS PER LITER	RATE OF STREAMING*		
	2 HOURS	24 HOURS	48 HOURS
gm.	*mm./min.*	*mm./min.*	*mm./min.*
10.000	0.3360	0.2473	0.1380
0.750	0.3660	0.3636	0.3468
0.500	0.4332†	0.4068	0.3756
0.250	0.3150	0.4596	0.4093
0.100	0.3890	0.4968†	0.4620†
0.075	0.3420	0.4356	0.3856
0.000	0.2385	0.2269	0.2424
Maximum percentage of increase	81.64	119.0	90.51

* Each figure represents the average of 50 readings.
† Maximum rate recorded.

The effect of ethylene chlorhydrin and of thiourea upon the rate of streaming of the protoplasm of *Nitella* is similar to their effect upon *Elodea*. In a graded series of concentrations of ethylene chlorhydrin, the rate of streaming of *Nitella* cells rises to a maximum at 0.25 gm. per liter after 2, 24, and 48 hours of treatment (table III). After 24 hours in a 10 gm. per liter solution of ethylene chlorhydrin the protoplasm of *Nitella*, like that of *Elodea*, has ceased to stream. The highest rate of streaming was obtained after a 24-hour treatment, although 2 hours produced almost as high a maximum rate.

TABLE III

NITELLA TREATED WITH ETHYLENE CHLORHYDRIN

CONCENTRATION IN GRAMS PER LITER	RATE OF STREAMING*		
	2 HOURS	24 HOURS	48 HOURS
gm.	*mm./min.*	*mm./min.*	*mm./min.*
10.000	1.494	0.000	0.000
0.750	2.310	1.963	1.925
0.500	1.971	1.946	2.130
0.250	2.669†	2.976†	2.241†
0.100	2.432	2.610	2.111
0.075	2.077	2.049	2.010
0.000	1.359	2.006	1.374
Maximum percentage of increase	99.24	48.37	63.1

* Each figure represents the average of 50 readings.
† Maximum rate recorded.

The effect of thiourea upon the rate of streaming of *Nitella* may be shown by a curve very similar to that of *Elodea*, the maximum rate occurring at 0.1 gm. concentration in both species (tables II, IV). As the concentration of the solutions increased, the rate of streaming decreased, but not to zero. The 2-hour treatment, however, showed a maximum in the curve at 0.075 gm., then decreased (table IV). Both ethylene chlorhydrin and thiourea increase the rate of streaming in *Nitella* in a shorter time than they do in *Elodea*.

TABLE IV

NITELLA TREATED WITH THIOUREA

CONCENTRATION IN GRAMS PER LITER	RATE OF STREAMING*		
	2 HOURS	24 HOURS	48 HOURS
gm.	*mm./min.*	*mm./min.*	*mm./min.*
10.000	0.923	1.224	1.744
0.750	1.305	2.158	1.957
0.500	1.385	3.052	2.246
0.250	1.164	1.792	2.152
0.100	1.639	2.600†	2.656†
0.075	1.813†	2.391	2.215
0.000	0.894	1.532	1.349
Maximum percentage of increase	102.8	69.72	96.87

* Each figure represents the average of 50 readings.

† Maximum rate recorded.

The fact that the rather acid distilled water (pH 5.6), used in making up the solutions, does not affect the streaming of protoplasm is shown by a comparison of readings taken from plants in distilled water with those taken from plants in tap water (table V).

TABLE V

ELODEA AND *NITELLA* IN WATER

PLANT	RATE OF STREAMING			
	TAP WATER	DISTILLED WATER		
		2 HOURS	24 HOURS	48 HOURS
	mm./min.	*mm./min.*	*mm./min.*	*mm./min.*
Elodea	0.2100	0.2616	0.2412	0.2208
	0.2004	0.2385	0.2269	0.2424
Nitella	1.894	1.5590	2.006	1.3740
		0.8940	1.532	1.3490

Discussion

The concentration of the solutions used in this experiment are necessarily far weaker than those used by DENNY (**4**) in his work on the potato, because of the more delicate nature of the material used in this experiment and the method of treating it. That the rate of streaming of the protoplasm of *Elodea* and of *Nitella* is increased by treatment with ethylene chlorhydrin and thiourea implies that these chemicals must change the protoplasm in some way. In the earlier work of SEIFRIZ (**16, 17, 18**), MAXIMOV (**10**), NADSON and MEISEL (**14**), NICHOLS (**15**), and WEBER (**19**) it was found that substances increasing the rate of streaming of protoplasm also decrease its viscosity, probably because they increase the dispersal of the colloidal particles in protoplasm. Further experimental work must be done before it is possible to say that the chemicals used in this experiment have this effect upon protoplasm, and in this way help to bring about the breaking of dormancy.

Summary

1. Both ethylene chlorhydrin and thiourea in concentrations of less than 1 per cent. and 5 per cent. respectively, after 24 and 48 hours' treatment, increase the rate of streaming of the protoplasm of *Elodea* and *Nitella* cells.

2. Concentrations of more than 1 per cent. ethylene chlorhydrin and 5 per cent. thiourea are toxic to *Elodea* and *Nitella*.

3. The maximum rates of streaming are produced by a solution of 0.01 per cent. ethylene chlorhydrin in *Elodea*, and of 0.025 per cent. in *Nitella;* and by a solution of 0.01 per cent. thiourea in *Elodea* and *Nitella*. The maximum increase in the rate of streaming of treated over untreated protoplasm averages 70 to 100 per cent.

The writer wishes to express her gratitude to Dr. HELEN A. CHOATE for suggesting the problem, and to Dr. CHOATE and Dr. DOROTHY DAY for their kind assistance in carrying out the work.

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BRIEF PAPERS

SPECTROGRAPHIC METHOD FOR DETERMINING THE CARBON DIOXIDE EXCHANGE BETWEEN AN ORGANISM AND ITS SURROUNDINGS[1]

(WITH TWO FIGURES)

The spectrographic method for the determination of carbon dioxide as here described makes use of the high opacity of CO_2 gas to radiation in one of its fundamental infra-red absorption bands. The spectral region used is a band from 4.2 to 4.3 μ. Two millimeters thickness of pure CO_2 at atmospheric pressure transmits only 22 per cent. of this radiation. This region was selected because water vapor and practically all other gases and vapors commonly present in air are transparent to this radiation.

Radiation of this wavelength is isolated from a suitable source (NERNST filament) by an infra-red spectrograph, and allowed to fall upon a vacuum thermocouple after passing through a known optical path length of air contained in an absorption tube. The apparatus is calibrated by filling the absorption tube with air free from CO_2, then with samples of known concentrations of CO_2, and the galvanometer deflections observed. For the range of concentrations from 0 to 0.1 per cent. CO_2 an optical path length slightly under 1 meter is used. With this arrangement the sensitivity of the apparatus is such that a 1-mm. deflection represents one ten-thousandth of 1 per cent. CO_2. For most work half this sensitivity is ample, and it is easier to hold constant. Shorter path lengths enable the measurement of greater concentrations.

The particular advantages of this spectrographic method are: (1) the sensitivity is as great as that of the best chemical method, (2) it gives instantaneous measurements, (3) the measurements are independent of the humidity of the air and also independent of the presence of practically all other gases or vapors commonly found in air, (4) for ordinary room temperatures and pressures the apparatus has very small temperature and pressure corrections, and (5) it can be recalibrated in a few seconds.

There are two factors in this method which limit the speed of response. One is the thermocouple-galvanometer system which by some sacrifice in

[1] A rapid and sensitive spectrographic method of determining the carbon dioxide concentration of the air surrounding small living organisms has been developed by Dr. E. D. MCALISTER, of the Division of Radiation and Organisms, Smithsonian Institution. It has proved so satisfactory in studying the carbon dioxide assimilation of young wheat plants that the Committee on Physical Methods deems it worth while to call attention to this unique method as a highly useful tool in studying some of the more intricate problems of photosynthesis and respiration of both plants and animals.—EARL S. JOHNSTON, *Chairman*.

sensitivity can be reduced to respond in about 1 second. The other factor is inherent in the growth chamber. In a closed system this depends upon how fast the air can be circulated from the plant or organism into the optical absorption tube to insure complete mixing. In a system devised for work on young wheat plants and provided with temperature and humidity control this was accomplished in about 20 seconds, as can be seen from figure 1. A

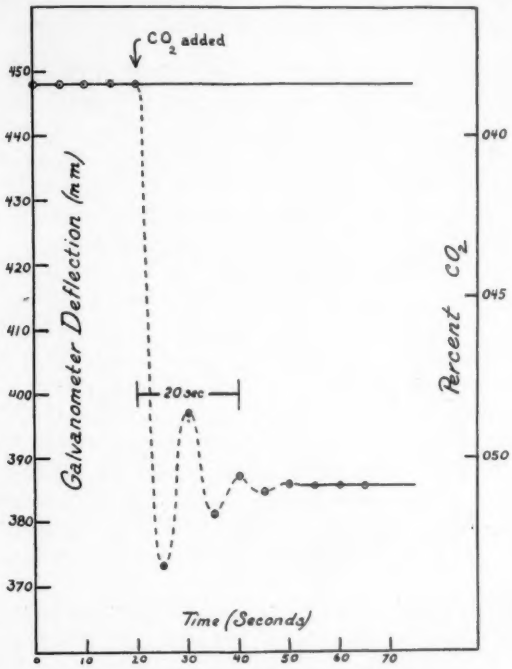

FIG. 1. Time response of the system.

small fan enclosed in the system causes the air to circulate once around the system in about 5 seconds. It can be seen from this figure that after about four trips around the system any change in CO_2 concentration has been effectively equalized.

The success of this method depends upon the use of a sensitive vacuum thermocouple and a high-sensitivity galvanometer. (Leeds and Northrup H. S. galvanometer with scale at 5 meters was used in this work.) Its reliability depends upon a steady output of radiation from the source and upon the "zero" of the galvanometer-thermocouple system. In the present setup a shutter operated by a synchronous motor enables the operator to read the deflection and the zero point every 30 seconds. This eliminates zero

drifts but keeps the operator busy. The apparatus can, of course, be made self-recording. The freedom from any effect due to changes in humidity of the air in the system was demonstrated by blowing, first, air nearly saturated with water through the system, next, by blowing the same air through after most of the water was removed by passing it through sulphuric acid and noting that no measurable deflection resulted.

Figure 2 shows a typical set of data taken on 6 one-week-old Marquis

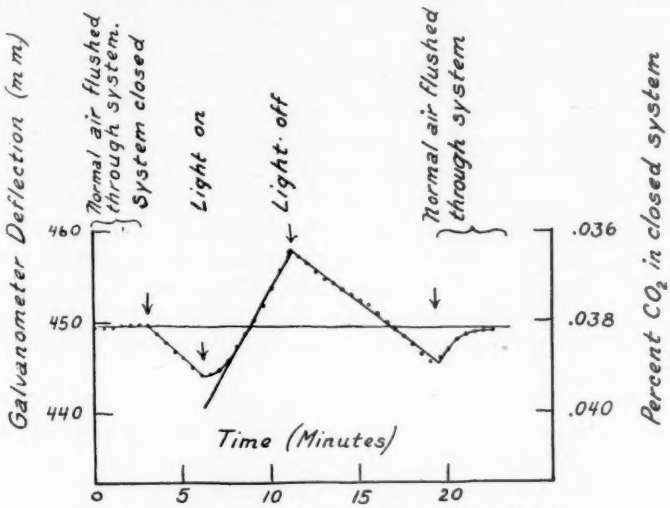

FIG. 2. Typical data on a green plant (Marquis wheat).

wheat plants. This shows how quickly the system follows the response of the plant. The short induction period seen in figure 2 during the first 2 minutes after illumination of the plants is surprisingly similar to that observed for algae by WARBURG, PAAUW, and others. It is to be noted, however, that by this method this induction period has for the first time been observed *directly* and not by the integration of the effect of a series of short exposures to light, each exposure being followed by a long period in darkness.—E. D. MCALISTER, *Smithsonian Institution, Washington, D. C.*

CAROTENOIDS OF THE PEACH[1]

The two main problems involved in determining the nature of the carotenoid complex in a given material are, first, a thorough extraction of the pigments, and second, separation of the various components. In the case of the peach, the carotenoid concentration is definitely low, when compared with leaves, or with other fruits such as apricots, tomatoes, or peppers. The problem is further intensified by the difficulties inherent in drying this fruit without decomposition of pigments, and by the hygroscopic nature of the dried material.

Through the courtesy of Dry Fruit Products Incorporated, of Oakland, the writer was furnished with powdered vacuum-dried peaches (Muir variety), of which approximately 45 per cent. passed a 60-mesh sieve, and only this portion was used in the extraction. The material absorbed some moisture as a result of exposure during sieving, weighing, and transfer operations, and its moisture content was between 4 and 5 per cent. Of the 60-mesh material 2.5 kilograms were steeped in acetone overnight. A second steeping showed that the carotenoids had been almost completely (90–95 per cent.) extracted, although the powder was still definitely colored with pigments which could be extracted with aqueous alcohol, but which were not transferable to ether or petroleum ether.

For purposes of comparison, 4.5 kg. of fresh Lovell peaches which had been placed in cold storage (0° F.) were dehydrated and extracted by the usual wet extraction method with acetone.

In both cases, the carotenoids were transferred to petroleum ether (B. P. 30°–70° C.), which was then thoroughly washed with water, dried and concentrated under reduced pressure.

Passage of the petroleum ether concentrates over a TSWETT column of magnesia by the method of STRAIN (5) showed only minor differences, possibly of a varietal nature, in the relative amounts of the carotenoid components present. The Muir extract contained sufficient pigment for more detailed examination.

Development of the chromatogram gave four major zones, with a minimum of eight constituent carotenoids. The colored zone, least strongly adsorbed, consisted of β-carotene, with a very faint trace of the α-component. This zone accounted for 35–40 per cent. of the total carotenoid coloring matter. Twelve mg. of recrystallized carotene were obtained from this zone. Absorption spectra maxima by the method of SMITH (3) in carbon bisulphide were 512 and 480 mμ, characteristic of β-carotene.

The second zone, from which 5 mg. of impure pigment were recovered,

[1] Contribution from the Division of Fruit Products, University of California, Berkeley.

accounted for approximately 25–30 per cent. of the coloring matter. It was identified as cryptoxanthin, absorption maxima in carbon bisulphide at 513 and 482 mμ. When mixed in equal proportions with cryptoxanthin from *Physalis alkekengi,* furnished by courtesy of DR. STRAIN, it behaved as a homogeneous fraction when adsorbed on magnesia. Addition of β-carotene caused the formation of two zones on another test column.

From the third zone were obtained two pigments in amounts of less than 2 mg. When partitioned between petroleum ether and 90 per cent. ethanol, they remained quantitatively in the petroleum ether phase. After treatment, however, by refluxing with alcoholic KOH for thirty minutes, the main portion could be extracted with 90 per cent. ethanol. The absorption maxima of these two pigments in carbon bisulphide and in ethanol were those of lutein and zeaxanthin, indistinguishable from α- and β-carotenes and their behavior toward solvents indicates that in the fruit they occur in an esterified condition.

The fourth and most strongly adsorbed zone, containing less than 10 per cent. of the color, on prolonged washing gave rise to three narrow bands. Absorption maxima were between those of lutein and zeaxanthin. Even after saponification, the pigments remained in the petroleum ether phase, when partitioned between this solvent and aqueous ethanol.[2]

Discussion

So far as the writer can ascertain, no examination of the carotenoids of the peach has been made, with application of the TSWETT column technique. The investigations of STRAIN (**6**) have shown that the leaf xanthophyll complex consists of at least a dozen xanthophyll components, and it is becoming increasingly apparent that an equally complex carotenoid fraction exists in other parts of the plant, and that only the more abundant components have been at all adequately characterized.

It is of interest that whereas the pigments of the apricot (**1, 2**), according to the work of KUHN and BROCKMANN, consist largely of β-carotene, γ-carotene, and lycopene (all hydrocarbons in composition), those of the peach are (apart from the β-carotene fraction) largely xanthophyll in nature. The results reported by L. L. W. SMITH and O. SMITH (**4**) may be readily interpreted in accord with this. Studying a different problem, the effect of light on the carotenoids of certain fruits, they found a low concentration of carotenoids (1.9 mg. per kg. flesh) in the peach compared with 21.7 mg. in the apricot. When light was excluded from the fruit by bagging, there was a marked increase in the carotenoid content of the peach, and an almost equally striking decrease for the apricot. Red-fleshed varieties of tomatoes also had a lower carotenoid content when bagged. The apricot and the tomato both

[2] In this respect they differ materially from antheraxanthin and petaloxanthin (**7**).

contain lycopene, whereas the peach apparently does not. It seems not improbable that the increase in carotenoid content of the bagged peach noted by SMITH and SMITH is due to an increase in the esterified xanthophyll fraction, and the decrease in the other fruits due to their failure to develop lycopene in the absence of light. In any event, their results indicate fundamental differences in the pigments of the peach and apricot.

Summary

Summarizing, we may conclude that the carotenoid fraction of the peach contains at least eight components. The four most abundant have been identified as β-carotene, cryptoxanthin, lutein, and zeaxanthin. Only a faint trace of α-carotene could be found. The vitamin A potency of the peach will of course, therefore, reside in the carotene and cryptoxanthin fractions. No trace of lycopene or of γ-carotene was detected, in which respect the carotenoid complex of the peach differs materially from that of the apricot.—
G. MACKINNEY, *University of California, Berkeley, California.*

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BORON CONTENT OF APPLES AT DIFFERENT STAGES OF DEVELOPMENT

In view of the current interest in boron as one of the elements essential for the normal growth of plants, and as a factor concerned in the occurrence of physiological disorders of the apple, the writers have determined the boron content of this fruit at various stages in its development. The material used was fruit of the variety Golden Russet originally collected by one of us (DeLong) for another purpose. This fruit was apparently normal in all respects and quite unaffected by any physiological disorders. Dates of collection and other descriptive data are given in table I. It should be noted that all of the samples are composite, approximately equal numbers of fruits having been gathered from each of several trees at each collection. These trees were of uniform age, all in a vigorous condition, and bore, in the season of collection, fair to good crops of fruit. The orchard soil is a sandy loam with a clay subsoil. The orchard is not cultivated, but the weed-grass growth is clipped in June. The chief fertilizer used is barnyard manure.

TABLE I

Date of collection	Laboratory number	Average fresh weight per apple	Dry weight of sample	No. of fruit in sample	Remarks
		gm.	*gm.*		
5/6/34	34–12	0.111	26.8	1000	Petals two-thirds fallen. Collected only fruit from which petals had dropped.
1/7/34	34–41	3.46	131.8	275	Starch beginning to appear in fruit.
31/7/34	34–59	36.79	313.2	60	
5/9/34	34–78	77.26	457.7	42	
	34–78A		119.4	42	This and succeeding "A" samples consist of the parings of the sample. Prior to this date the fruit was ashed without paring.
1/10/34	34–109	102.2	603.0	40	
	34–109A		117.3	40	
15/10/34	34–111	108.2	652.3	40	
	34–111A		113.1	40	

The analytical samples consisted of forty-gram portions of the dried tissue in each case, except that of no. 34–12, in which the total dry weight amounted only to 26.8 grams, and the whole amount was taken. These were

ashed in the presence of an excess of ten per cent. potassium carbonate solution at a temperature of about 450° C. with lixiviation. The method of analysis of the solutions so obtained was that described by SMITH.[1] The results obtained are given in table II.

These data indicate that in the healthy apple fruit the boron content progressively increases throughout the season. This increase is relatively very rapid for the period of active cell division and rapid growth, during the month of June, after which the rate of accumulation of this element becomes very much slower, although accumulation continues up to the time when the fruit is normally picked. In terms of parts per million of dry weight of tissue, however, the boron content shows a fairly rapid decrease during June and July, after which the amount present remains practically constant. When considered from either of the above viewpoints these results emphasize the apparent importance of boron during the period when rapid growth of the fruit is taking place. The epidermal tissues, as represented by the parings, do not seem to vary appreciably in boron content from the flesh of the fruit, although they are possibly somewhat higher.—J. C. JOHNSON and W. A. DELONG, *Acadia University, Wolfville, Nova Scotia.*

TABLE II

LABORATORY NUMBER	BORON PER APPLE	BORON, DRY WEIGHT BASIS
	mg.	*p.p.m.*
34–12	0.00087	33
34–41	0.0104	22
34–59	0.079	15
34–78	0.140	13
34–78A	0.043	15
34–109	0.178	12
34–109A	0.044	15
34–111	0.212	13
34–111A	0.043	15

[1] SMITH, G. S. The determination of small amounts of boron by means of quinalizarin. Analyst 60: 735–739. 1935.

NOTES

Thirteenth Annual Meeting.—The thirteenth annual meeting of the American Society of Plant Physiologists was held at Atlantic City, December 29–31, 1936. The meeting was a very interesting and valuable one, and the program committee deserves great commendation for the manner in which a difficult situation was handled. The problem of accommodating the papers offered to the committee by a rapidly expanding membership becomes more severe every year. It is probable that the meetings will have to be extended to four days, or that the number of joint meetings held with other groups may have to be reduced if all papers are to be placed upon the program. The American Society for Horticultural Science has found a method of handling a very large number of papers in record time—138 papers in one day! They met in four sections with simultaneous meetings, and divided their papers into those to be presented by the authors, and those to be *read on demand*. One can imagine that the demand was not very great, with so many papers being presented in person! There may be much in this technique worthy of consideration by other groups harassed by too many papers for the short meeting periods.

The outstanding features of the program were the symposia on mineral nutrition including the minor elements, and on carbon dioxide assimilation. It is obvious, from comments overheard at the meeting, that programs of this type are of the greatest value to those who attend them.

The annual dinner was held on Tuesday evening, December 29, at the Hotel Dennis. The retiring president, Dr. A. E. MURNEEK, was unable to be present, owing to illness, and his address on recent advances in the physiology of reproduction in plants was read by Dr. OTIS F. CURTIS, vice-president of the Society. Other features of the dinner included the fifth award of the STEPHEN HALES prize, and the twelfth award of the CHARLES REID BARNES life membership, details of which are noted later. Two members of the society were elected as patrons in accordance with the constitutional provision for those who have contributed sufficiently to the financial support of the society. The secretary-treasurer made a very encouraging report which included the information that the society had passed the 500 mark in membership, and that library subscriptions had been notably increased during the year.

Atlantic City redeemed itself as a convention city in mid-winter. In 1932, the entire week was rainy; but the 1936 meetings occurred under almost ideal weather conditions. Only one day was rainy, and the week opened with weather warm and sunny enough for a summer day. Every-

thing considered, this meeting was one of the most satisfying ones in the history of the organization.

Life Membership Award.—The CHARLES REID BARNES life membership award was established at the Kansas City meeting in 1925, the second annual meeting of the American Society of Plant Physiologists, and the first award was made at Philadelphia in 1926. At the Atlantic City meeting in 1936, the twelfth award of this honor was made to Dr. DANIEL TREMBLY MACDOUGAL, pioneer among American plant physiologists, whose long career as Director of the Laboratory of Plant Physiology of the Carnegie Institution of Washington has been an inspiration to everyone. He published an early text book on plant physiology, second in point of time in America only to GOODALE's text, and in this way exerted a profound influence upon the development of experimental plant physiology in this country. He was a contemporary of Dr. BARNES, about six years younger than BARNES, and they were close friends. Dr. MACDOUGAL's contributions to the literature of plant physiology have been numerous, and are so well and favorably known that they need no enumeration. The society is to be congratulated upon the selection, and the committee of award, under the leadership of Dr. JOHN W. SHIVE, deserves great commendation for having exercised for the first time its highest constitutional privileges.

Stephen Hales Award.—The fifth award of the STEPHEN HALES prize was made to Dr. KENNETH V. THIMANN, formerly of the California Institute of Technology, now at Harvard University, for his contributions to our knowledge of the chemistry and physiological significance of the growth hormones of plants. He has also been a contributor of notable papers in the field of protein chemistry. Dr. THIMANN was born in England in 1904, and began to contribute actively to our knowledge of proteins at the age of 22. He received his training and degrees at the Imperial College, University of London (B.Sc., 1924; Ph.D., 1928), and distinguished himself as Frank Hatton prizeman in Chemistry in 1924. Later he was a demonstrator at the University of London, and held a Beit Memorial Research Fellowship in biochemistry from 1927 to 1929. In 1930 he was invited to join the staff of the William G. Kerckhoff Laboratories of the California Institute of Technology at Pasadena. Here he began his brilliant contributions to the field of plant hormones.

In the absence of the chairman of the STEPHEN HALES award committee, Dr. W. W. GARNER of the United States Department of Agriculture, the candidate was presented for the award by Dr. CHARLES A. SHULL. The certificate of award and the prize were conferred upon Dr. THIMANN by President R. B. HARVEY. A delightfully informal story of the career of

Dr. THIMANN was presented to the Society by Dr. GEORGE S. AVERY of the Connecticut College for Women.

Rodney Howard True.—In celebration of the seventieth anniversary of his birth, this number of Plant Physiology is dedicated to Dr. RODNEY HOWARD TRUE, who has been for many years professor of botany, director of the Botanic Garden, and Director of the Morris Arboretum at the University of Pennsylvania. Dr. TRUE was born at Baraboo, Wisconsin, October 14, 1866, won his earlier degrees at the University of Wisconsin, and his Ph.D. at Leipzig in 1895. He began his work in pharmacognosy at the University of Wisconsin, was lecturer at Harvard for a short time, and then plant physiologist in charge of physiological investigations in the Bureau of Plant Industry from 1902 to 1920. He went to the University of Pennsylvania from the U. S. Department of Agriculture in 1920. His contributions to our understanding of the relationships between root systems and the solutions in which they grow have been fundamentally very valuable. In other lands the "Festschrift" has been developed as a means of celebrating the seventieth birthday anniversary of those whose lives have been devoted to scientific research. The American Society of Plant Physiologists is happy to dedicate this number of PLANT PHYSIOLOGY to Dr. TRUE in celebration of his seventieth birthday anniversary, reached on October 14, 1936. With the dedication go the good wishes of all that Dr. TRUE may have many more years of happy and satisfying activity.

Finance Committee Report.—During the year 1936, two of the bonds possessed by the American Society of Plant Physiologists were called for redemption. One of these was a Texas Corporation bond, the other a gold debenture of the American Telephone and Telegraph Co. The funds rendered liquid by these calls have been reinvested, partly in a Texas and Pacific R. R. general and refunding bond, and partly in shares of Massachusetts Investor's Trust. The purchase of the latter investment was dictated by the present unhealthy condition of the bond market, and by the need of a hedge against a moderate inflation which has by no means yet run its course.

The Society's permanent funds amount to nearly $8,000, with $2,000 in the STEPHEN HALES endowment, $3,000 in the CHARLES REID BARNES fund, $1,400 in life memberships, slightly less than $500 in the general endowment, and securities of unknown value in the reserve, represented by $1,100 in real estate gold bonds. The first several funds are adequate to support their objectives at present, but the general endowment should be increased fifty-fold in order to contribute substantially to the publication program.

Abstracts.—Occasionally authors have failed or refused to submit abstracts of their papers appearing in PLANT PHYSIOLOGY. These abstracts are collected by the editor for use in *Biological Abstracts*. As long as this cooperation is needed by *Biological Abstracts*, it is the duty of authors to respond with an abstract typewritten on the blanks sent out with the galley proofs. The task of editing the papers used in PLANT PHYSIOLOGY has become so large that there is no time that can be devoted to writing repeatedly to delinquent authors. To clear this situation, the editor proposes to place with the printers of the journal more papers than are currently needed for each issue. When the forms must be closed, the issue will be made up of those papers whose authors have complied with the abstract requirement. The editor does not wish to assume the duty of even reminding authors that they have not sent an abstract. The abstract blank is supposed to be enclosed with the proofs, and is sufficient notice that it should be returned along with the corrected galleys.

Methods in Plant Physiology.—A laboratory manual and research handbook of methods in plant physiology was published on January 1, 1937, by the McGraw-Hill Book Co., New York. The authors are Dr. W. E. LOOMIS, Iowa State College, and Dr. C. A. SHULL, University of Chicago. One chapter on statistical methods was contributed by Dr. GEORGE W. SNEDECOR, Iowa State College.

The first part of the book contains thirteen chapters, twelve of which are devoted to large sections of plant physiology, arranged in logical order. There are 182 experiments in all, and each experiment bears a designation to indicate whether it is elementary in character, intermediate, or advanced. The work can be adapted, therefore, to beginners, as well as to intermediate students, and to advanced or graduate students. The work can be made to serve as a laboratory manual at any level of training desired.

The second part is devoted particularly to methods useful in preparing for research in plant physiology. There is a chapter on general procedures, followed by six chapters dealing with analytical work. The first of these considers the collection of samples, killing, preserving, and extracting plant materials. Then follow chapters on the soluble carbohydrates, colloidal carbohydrates, soluble nitrogen, colloidal nitrogen, and plant ash. There is a chapter on physical measurements, and one on the measurement and control of environment. The final chapter is on statistical methods. An appendix provides 33 tables useful to the physiologist in his calculations. There are 472 pages, with the index, and the publisher's price is $4.50 per copy.